PROCEEDINGS

OF THE

SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

VOL. 32. NOVEMBER, 1934. No. 2.

Illinois Section.

University of Chicago, October 23, 1934.

7635 P*

Influence of Thyro-parathyroidectomy and of Parathyroid Hormone upon State of Calcium in Serum of the Cat.†

FRANKLIN C. MCLEAN, B. O. BARNES AND A. BAIRD HASTINGS.

From the Physiological Laboratories and the Lasker Foundation for Medical Research, University of Chicago.

Trendelenburg and Goebel[1] demonstrated that serum from thyro-parathyroidectomized cats, when used as a nutrient fluid for the isolated heart of the frog, diminished the amplitude of contraction of the heart, as compared with serum from normal cats. They attributed this effect to a reduction in the total calcium of the serum, with a corresponding decrease in ionized calcium, but were unable to rule out the possibility of the formation of an un-ionized compound of calcium, presumably by combination with an organic acid.

Two of the authors have shown that the sensitivity of the frog's heart to changes in the concentration of calcium is specific for ionized calcium, bound calcium being inert with respect to the preparation, and have utilized this property of the heart for direct quan-

* P represents a preliminary, C a complete manuscript.

† This work was aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Trendelenburg, P., and Goebel, W., *Arch. f. exp. Path. u. Pharm.*, 1921, **89**, 171.

titative estimations of Ca^{++} concentrations in biological fluids.[2] They have also shown[3] that the ionization of calcium in the protein-containing fluids of the human body follows, as a first approximation, a simple mass-law relationship, expressed by the equation

$$(1) \qquad \frac{[Ca^{++}] \times [Prot^{=}]}{[Ca\,Prot]} = K = 10^{-2.22}$$

from which it is possible to calculate Ca^{++} concentrations, from analysis for total calcium and total protein, with a degree of accuracy at least as great as that of direct observation by the frog heart method.

Using the methods of direct observation and of calculation of Ca^{++} concentrations, the authors have studied the influence of thyroparathyroidectomy and of the administration of the parathyroid hormone upon the ionization of calcium in the serum of the cat. The cat was chosen as the experimental animal because its serum was found to be especially favorable for observations by the frog heart method, which is not the case with the dog, and because the state of calcium in its serum was found to be accurately described by the mass-law relationship above referred to, which is not the case with the rabbit. The cat was found, in every instance, to respond to administration of the parathyroid hormone with a typical rise in the serum calcium level, resulting, in some cases, in death following the symptoms of hyperparathyroidism. A wide variation, however, was found in the rapidity of the response of cats to uniform daily doses of parathyroid extract. This variability, which may account for the conflicting statements in the literature[4] as to the response of the cat to the parathyroid hormone, is being further investigated.

In more than 50 observations upon 9 cats the mass-law relationship previously described for normal human serum has been found to hold for the cat over the entire range from hypo-calcemia with tetany to the maximum hypercalcemia induced by administration of the parathyroid hormone. The values for the constant in the mass-law equation were, within the limits of experimental error, the same as those found for normal human serum, and there was good agreement between observed and calculated values for Ca^{++} concentrations over the entire range. Since on account of the limitations of the frog heart method, it was not possible to make direct observa-

[2] McLean, F. C., and Hastings, A. B., *J. Biol. Chem.*, 1934, **107**, 337.

[3] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 529.

[4] Thomson, D. L., and Collip, J. B., *Physiol. Rev.*, 1932, **12**, 309.

tions of Ca^{++} in undiluted hypercalcemic serum, observations upon these sera were made upon diluted serum only. Diluted hypercalcemic serum from the cat was found to conform to the mass-law Equation 1, as is also the case with diluted normal serum from the cat and from man.

In summary, the hypo-calcemia following thyro-parathyroidectomy results in a correspondingly diminished concentration of Ca^{++} and the hyper-calcemia following administration of the parathyroid hormone results in a correspondingly increased concentration of Ca^{++}. No evidence of any effect of removal of the parathyroid glands, or of administration of the parathyroid hormone, upon the qualitative state of calcium in the serum has been found, the changes observed being simply the quantitative changes predicted by the mass-law Equation 1.

Symptoms of tetany have usually appeared at a Ca^{++} concentration of approximately 0.6 mM per kg. H_2O, have increased in severity at still lower levels, and have disappeared as the Ca^{++} concentration rose again above the 0.6 mM level. Of 7 cats operated upon, 6 have developed tetany, the lowest Ca^{++} concentration observed being 0.40 mM per kg. H_2O (0.43 calculated), accompanying severe tetany, with a total calcium concentration of 1.04 mM per liter. The normal Ca^{++} range in the serum of the cat may be put tentatively at from 1.05 to 1.25 mM per kg. H_2O, and the level at which death from hyper-parathyroidism may occur at from 1.7 to 2.0 mM per kg. H_2O.

7636 P

Peristaltic Effect of Stimulation of the Chorda Tympani.*

H. NECHELES, R. FRANK AND L. ZIMMERMAN.

From the Gastro-Intestinal Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

Dixon,[1] Dale[2] and Loewi[3] originally established acetylcholine as the humoral agent that produces motility of the gastro-intestinal tract. Later research, recently summarised by Cannon[4] and Dale[5]

* Aided by the Louis L. Cohn Fund.

1 Dixon, W. E., *Brit. Med. J.*, 1906, **2**, 1807.

2 Dale, H. H., *J. Pharm.*, 1914, **6**, 147.

3 Loewi, O., *Pflüger's Arch.*, 1921, **193**, 201.

4 Cannon, W. B., *Am. J. Med. Sciences*, 1934, **188**, II:145.

5 Dale, H. H., *Brit. Med. J.*, 1934, **1**, 835.

has shown that acetylcholine is liberated by stimulation not only of parasympathetic nerves and sympathetic ganglia, but probably even with stimulation of striated muscle. Fröhner[6] reported that eserine was commonly used to produce increased peristaltic activity of the gastro-intestinal tract in animals; and since the work of Hunt,[7] Fühner[8] and Simonart,[9] it has been known that eserine added to acetylcholine greatly fortifies the action of acetylcholine. Matthes[10] and Loewi and Engelhart[11] have added to our knowledge that eserine prevents the hydrolysis of acetylcholine by blood and tissues through inhibiting an esterase. In the light of this knowledge, the authors investigated the effect of eserine and acetylcholine on ileus following peritonitis (to be published). In this research it was found that in peritonitis acetylcholine given intramuscularly in small doses produced either no effect or a very small one, but it was always effective when given with eserine. The present research was undertaken to investigate whether or not the stimulation of salivary secretion would produce motility of the intestine through liberation of acetylcholine from the stimulated salivary glands. Babkin, Alley and Stavraky[12] have demonstrated that acetylcholine is liberated into the venous blood when the chorda tympani was stimulated.

Healthy dogs under light ether anesthesia were used. Both chordae tympani were prepared and stimulated with weak faradic currents from a Harvard inductorium. Balloons were placed in the stomach, ileum and upper part of the colon, and connected with water manometers. Blood pressure was recorded in the usual way. The effect of stimulation of the chordae tympani was checked by observing or recording the salivary flow from cannulas inserted in Wharton's ducts. Eserine salicylate was used, 1 mg. per 10 kg. of body weight being injected intramuscularly or intravenously.

In normal animals it was found that eserine (0.5-1.0 mg. intravenously or intramuscularly) had a powerful effect on gastro-intestinal motility, but that this action was inconstant in occurrence and in magnitude. Thus in 2 animals there was no effect, while in 3 animals there was increased motility. Stimulation of the

[6] Fröhner, E., *Lehrbuch der Allgemeinen Therapie fuer Tieraerzte ed. Stuttgart, Ferd. Enke,* 1900.

[7] Hunt, R., and Renshaw, R. R., *J. Pharmacology exp. Therap.*, 1934, **51**, 231.

[8] Fühner, H., *Arch. Exp. Path. u. Pharmac.*, 1918, **82**, 51, 205.

[9] Simonart, A., *J. Pharm.*, 1932, **46**, 157.

[10] Matthes, K., *J. Physiol.*, 1930, **70**, 338.

[11] Loewi, O., and Engelhart, E., *Arch. f. exp. Path. u. Pharmac.*, 1930, **150**, 1.

[12] Babkin, B. P., Alley, A., and Stavraky, G. W., *Trans. Roy. Soc. Can.*, 1932, **26**, 89.

chordae tympani after the administration of eserine regularly produced gastro-intestinal motility. Mere stimulation of the chordae tympani likewise produced motility of the small intestine and the colon. In all 4 dogs in which stimulation of the chordae tympani was performed without previous injection of eserine, motility of the ileum and colon was obtained (Fig. 1). It appears that factors are

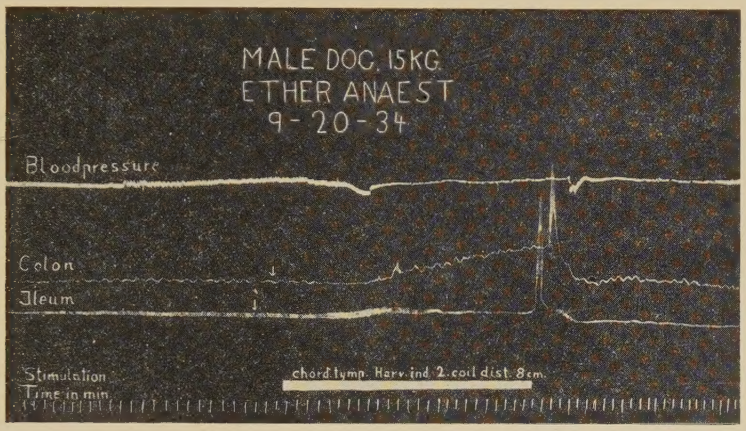


FIG. 1.
The sudden elevation of the curves of the ileum and colon at the end of stimulation of the chorda is due to panting of the dog.

present in the blood which alter the activity of the esterase allowing the acetylcholine liberated by stimulation of the chordae tympani to affect more or less the gastro-intestinal tract and to produce varying degrees of motility. This phase of the investigation is being continued.

7637 C

Total Versus Extra Oxygen Consumption in Muscle Activity in Relation to Fiber Length.

PAUL W. SMITH. (Introduced by M. B. Visscher.)

From the Department of Physiology, College of Medicine, University of Illinois, Chicago.

It appeared to the author that it would be of considerable importance if the fundamental behavior of cardiac and skeletal muscle could be more closely correlated by the explanation of an apparent

difference in their behavior following changes in initial fiber length.

Evans and Hill[1] showed that the heat produced during a short tetanus of the frog's sartorius increases as the muscle is extended rising to a maximum at about the *in situ* resting length, and then decreases upon further extension.

Starling and Visscher,[2] utilizing the isolated heart-lung preparation, showed that the oxygen consumption of the mammalian ventricle rises with increasing diastolic volume, but found no indication of a maximum with subsequent decline, although they explored a very wide range of alterations in ventricular volume. It is obvious that these experiments involved the measurement of the total oxygen requirement of the heart, while in the myothermic experiments quoted above only that excess metabolism due directly to activity was determined.

Feng[3] reported that the resting heat production and oxygen consumption of the frog's sartorius both rise with increase in the length of the muscle. These observations might have been anticipated in the light of the experiments of Clark and White,[4] who showed that the oxygen consumption of the quiescent, filled auricle of the cold blooded heart approached that of the beating, empty auricle.

Frog's sartorii were first measured *in situ* with the limbs in a uniform position, then excised and again measured at the minimum length which they would assume, floating freely in Ringer's fluid. They were soaked in Ringer for 3 hours and their oxygen consumption measured in a microrespirometer of the Thunberg-Fenn type. The respirometer used embodied 2 convenient modifications. The 2 chambers were connected by capillary tubing and a stopcock which could be opened to permit the introduction of oxygen through the apparatus at any time without removing it from the bath. The inserts or stoppers of the muscle chambers consisted of an outer tube continuous with the ground glass stopper, and an inner tube bearing the sealed-in electrodes. The space between was sealed with petrolatum. The muscle was suspended between two lugs or projections, one at the lower end of the inner tube, the other on the outer jacket just below the stopper. This arrangement permitted longitudinal adjustment of the inner tube, and therefore changes in the length of the muscle without removing the apparatus from the

[1] Evans, C. L., and Hill, A. V., *J. Physiol.*, 1914, **49**, 10.

[2] Starling, E. H., and Visscher, M. B., *J. Physiol.*, 1927, **62**, 243.

[3] Feng, T. P., *J. Physiol.*, 1932, **74**, 441.

[4] Clark, A. J., and White, A. C., *J. Physiol.*, 1929, **68**, 406.

constant-temperature bath. The inner tube was immobilized by means of a short sheath of heavy rubber tubing surrounding both the inner tube and the top of the outer jacket, thus preventing movement during contraction of the muscle.

The muscle was placed in the respirometer at its shortest length and the resting oxygen consumption measured. Ten maximal, break-induced shocks were then delivered directly to the muscle at a rate of 1 per second, and the excess oxygen requirement due to the isometric contractions thus determined. When the oxygen consumption had returned to the original resting rate, the muscle was extended by regular steps, usually 3 to 4 mm., and both resting and activity oxygen usage determined at each length. A control determination was made by returning the muscle to its first length at the end of the experiment.

The results of a typical experiment are presented in Fig. 1. The activity oxygen consumption was found to rise to a maximum at a length of 124% of the minimum length (100%) and to decline upon a further extension to 136.5% of the minimum length. The resting oxygen consumption continued to rise upon extension with an actual increase in steepness of the curve beyond the 124% length. This upturn in the resting oxygen usage was a quite uniform finding. Since muscles returned to their original length at the end of the

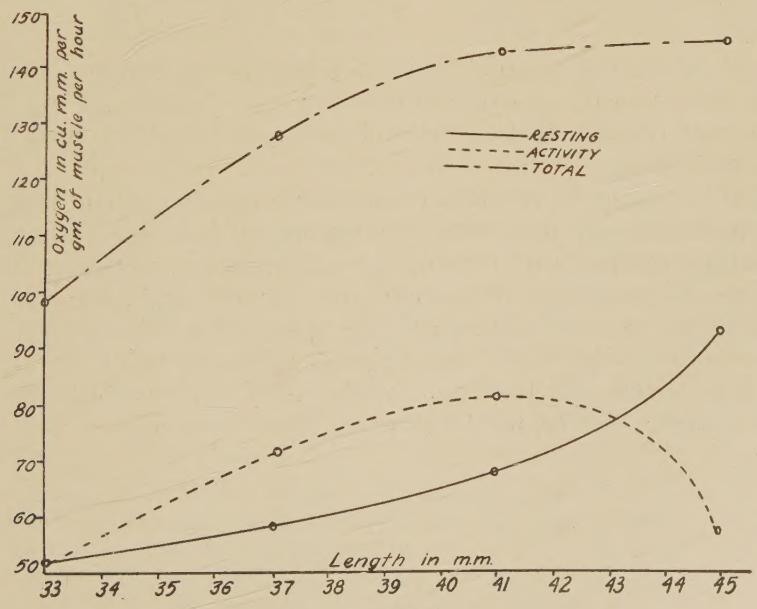


FIG. 1

experiment returned also to approximately their original oxygen usage, it is not believed that this is an indication of actual injury.

The *total* oxygen requirement continued to increase with extension of the muscle, in the majority of experiments approximating closely a straight line. Fig. 2 presents the protocol of this experiment, showing the actual capillary readings in millimeters of movement in 10 minutes plotted along a time axis.

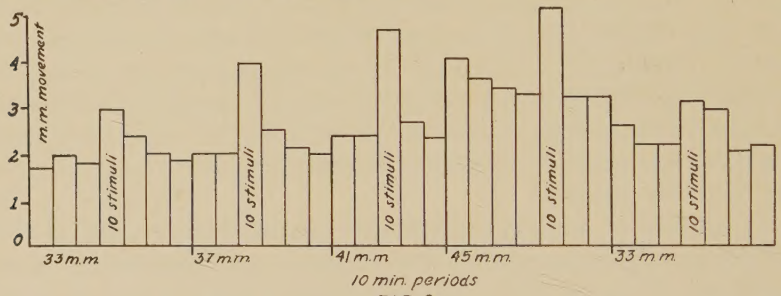


FIG. 2

TABLE I.

Experiment No. 1. Length *in situ*, 39 mm., on Ringer 35 mm. Weight, 0.100 gm. Temperature, 25° C. Oxygen in cu. mm. per gram of muscle per hour.

Length in mm.	36	39	42	46	39
Resting O_2	48	51	54	87	48
Activity O_2	57	63	66	45	
Total O_2	105	114	120	132	

In Table I are given the oxygen consumption data from a second typical experiment, in which the total oxygen requirement follows a perfectly straight line up to an extension of 28.5% over the minimum length.

It would appear from these data that the response of both cardiac and skeletal muscle to increase in fiber length is, in fact, identical, since that portion of the metabolism not directly associated with recovery from activity becomes an increasingly significant part of the total metabolism at appreciably increased initial fiber length.

7638 P

Effects of Constriction and Release of an Extremity.

HARWELL WILSON AND NORMAN W. ROOME. (Introduced by D. B. Phemister.)

From the Department of Surgery, University of Chicago.

The dangers of prolonged tourniquet application are as follows: 1. Interference with nutrition of the part sufficient to cause gangrene; 2. Paralysis, possibly due to prolonged pressure on the nerves; 3. Circulatory depression which frequently occurs after the release of a constrictor. Paolucci[1] and Fogliani[2] observed in animals that this circulatory depression was followed by death in many cases.

The present study is an attempt to evaluate the possible factors producing this fall of blood pressure and death. The factors considered were: 1. Return of blood to the limb (if this had been expressed before application of the tourniquet); 2. Reactive hyperemia (Bayliss); 3. Absorption of toxic metabolites; 4. Swelling of the limb by transudation.

Experiments were done on 19 dogs anesthetized with sodium barbital. One hind leg was constricted at the level of the hip joint for periods of from 2 to 20 hours. Six animals recovered after tourniquet applications of 2 to 6½ hours while 13 dogs died after constrictions of 3 to 20 hours. Seventeen percent of a control series of dogs died from the prolonged anesthesia.

In the animals dying, the hind quarters were symmetrically removed after the method of Blalock.[3] There was an average increase in weight of the ligated limb of 3.54% of the body weight. Plasma protein estimations showed the edema fluid to resemble diluted blood plasma. Johnson and Blalock[4] found that a loss of plasma equal to 2.4% of the body weight in 28 hours was sufficient to cause the death of dogs. In 3 cases gas was present in the tissues; clostridia were found in a high percentage of cases from both the normal and the ligated leg. These were not tested for exotoxin production and their identity with *B. welchi* was therefore not definitely established (Trusler and Reeves[5]).

---

1 Paolucci, R., *Arch. Ital. Di. Chir.*, 1928, **21**, 229.

2 Fogliani, U., *Rivista di Patologia Sperimentele*, 1932, **9**, 257.

3 Blalock, A., *Arch. Surg.*, 1930, **20**, 959.

4 Johnson, G. S., and Blalock, A., *Arch. Surg.*, 1931, **22**, 626.

5 Trusler, H. M., and Reeves, J. R., *Arch. Surg.*, 1934, **28**, 479.

Further studies are necessary to definitely determine the rôle of bacterial products in the causation of death; however, since the loss of plasma-like fluid into the ligated leg was adequate to produce death this would seem to be the most likely explanation.

These findings agree with studies in traumatic shock which show the serious consequences of a decrease of the circulating blood volume as a result of local fluid loss into the tissues.[3, 6]

7639 P

Effect of Dinitrophenol, Sodium Citrate, Sodium Bicarbonate, and Citric Acid upon Distribution of Cholesterol.

IRWIN C. WINTER, DANIEL HALE AND CHESTER J. FARMER.

From the Department of Chemistry, Northwestern University Medical School, Chicago.

The report of Marie[1] that sodium citrate administered by mouth or intravenously reduced the blood cholesterol of rabbits 60% led us to try its effect upon blood cholesterol of other species. It is of interest in this connection to note that dogs with a hyperpyrexia induced by the administration of 10 mg./kg. dinitrophenol showed an increase in blood cholesterol, the initial value of 161 mg. % being elevated to 285 mg. % 2 hours after injection of the drug. These observations led us to continue the study with albino rats.

Five groups of 5 rats each were placed upon a diet similar to that suggested by Chanutin and Ludewig,[2] in which extracted casein was substituted for extracted dried beef. To this cholesterol deficient diet, cholesterol was then added to the extent of 2%. The first group of rats received no supplement and served as control animals. In the second group, each animal received a daily subcutaneous injection of 1 mg. dinitrophenol per 100 gm. body weight. To the third, 0.2 gm. sodium citrate per 100 gm. body weight was given in aqueous solution by stomach tube. The fourth and fifth groups received $NaHCO_3$ and citric acid solutions, respectively, by stomach tube, in order to determine, if possible, the ion responsible for any change in the distribution of cholesterol. These 2 substances were given in amounts furnishing separately the same quantities of

[6] Roome, N. W., Keith, Wm. S., and Phemister, D. B., *S. G. O.*, 1933, **56**, 161.

[1] Marie, A. C., *Compt. rend. Soc. Biol.*, 1932, **109**, 13.

[2] Chanutin, A., and Ludewig, A., *J. Biol. Chem.*, 1933, **102**, 57.

TABLE I.

Series	Control			Dinitrophenol			Sodium Citrate			$NaHCO_3$			Citric Acid		
Range	Av.	low	high	Av.	low	high	Av.	low	high	Av.	low	high	Av.	low	high
Blood mg./100 cc. plasma (Blood of each group pooled for analysis)															
Total chol.	93			108			90			95			77		
Free ''	29			28			23			28			26		
Ester ''	64			80			67			67			51		
Total F. A.	474			431			522			431			284		
Lipoid P	6.07			7.47			6.47			6.17			5.64		
Livers mg./100 gm. body weight															
Total chol.	57	40	63	44	21	50	58	41	65	89	65	101	57	40	61
'' F. A.	247	230	268	198	162	210	265	241	274	357	335	380	240	225	270
Sterols mg./100 gm. body weight															
Carcass	337	326	340	300	285	310	319	305	335	306	285	319	287	251	299
'' + liver	394	383	405	344	320	360	377	360	389	395	380	409	344	321	363
Feces mg./100 gm. body weight (Feces of each group mixed and analyzed as group)															
Sterol	626			773			557			678			633		
Neutral fat	29			37			15			88			84		

sodium and citrate ions as received by the animals in Group 3 on the sodium citrate supplement. Each animal of the fourth group, therefore, received 0.14 gm. $NaHCO_3$, and of the fifth group 0.11 gm. citric acid daily, per 100 gm. body weight. At the end of 3 days the rats were killed, the blood from each group pooled and analyzed for total and free cholesterol, total fatty acids, and lipoid P. The livers were removed and analyzed individually for total cholesterol and total fatty acids, the carcass likewise for total sterols, and the feces for free fatty acids, sterols, and neutral fat. The cholesterol of the blood and liver was determined by means of the Yasuda modification of Okey's method,[3] while for fatty acids Bloor's oxidative method[4] was employed. Lipoid phosphorus was estimated by a modification of the Kuttner-Lichenstein method.[5] The carcass sterols were determined as non-saponifiable material, and the feces were analyzed as described by Gregory and Drummond.[6] The results, calculated on the basis of 100 gm. body weight and averaged for each group, are given in Table I. The range of values obtained by those analyses carried out separately on each animal (*i. e.*, livers and carcass) are also included in the table.

The dinitrophenol increases the cholesterol esters of the blood and reduces the cholesterol of the liver and carcass, while the excretion of sterol is increased. The fatty acids of the blood and liver are reduced slightly. The lipoid P of the plasma is increased.

Sodium citrate has very little effect on the cholesterol of the blood and liver, but reduces the sterol of the feces, and to a slight extent the sterol of the carcass. An increase occurred in the fatty acids of the blood. Our work on the rat is, therefore, not in conformity with the observations of Marie on the rabbit.

$NaHCO_3$ has no effect on blood cholesterol, but increases the sterol of the liver and feces. The carcass sterol is somewhat lowered, but the total sterol (carcass and liver) is unchanged, indicating a shift to the liver. The fatty acids of the blood are reduced, but increased in the liver. Neutral fat excretion is increased.

Citric acid decreases plasma ester cholesterol, fatty acids, and lipoid P. It has no effect on the cholesterol of the liver. The carcass sterol is reduced, but the sterol excretion is unaffected. The neutral fat of the feces is increased. The constancy of free cholesterol of blood is worthy of note.

---

[3] Yasuda, M., *J. Biol. Chem.*, 1931, **92**, 303.

[4] Bloor, W. R., *J. Biol. Chem.*, 1928, **77**, 53.

[5] Farmer, C. J., and Winter, I. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, To be published shortly.

[6] Gregory, E., and Drummond, J. C., *Z. für Vitaminforschung*, 1932, **1**, 257.

7640 C

Comparative Observations on Bacteriolytic and Hemolytic Titers of Certain Sera.

EVELYN B. TILDEN. (Introduced by Arthur I. Kendall.)

From the Department of Research Bacteriology, Northwestern University Medical School.

The Bordet Gengou reaction was designed to test the hypothesis of the identity of bacteriolytic and hemolytic complement,[1] although Bordet recognized later that the identity could not be regarded as absolute, since he was able to make specific anticomplements for the sera of various animals.[2] Muir and Browning[3] suggested that the bactericidal action of normal serum might be due to some moiety of complement which they termed "bacteriophilic". Gordon and his coworkers[4] have been unable to obtain a serum which is devoid of hemolytic complement and yet possesses bactericidal action, though the reverse might be true of sera absorbed with bacteria, unless absorption were continued too long or the number of bacteria used were overwhelming.

A series of tests was made to determine which bacteria in the School collection could be depended upon to resist the bactericidal action of certain non-immune sera, and which bacteria were sensitive to it. The results with pooled human sera did not vary, but occasionally an organism was killed by some rabbit sera and not by others (*e. g., B. enteritidis, B. typhosus* Rawlins). Table I is a resumé of these tests.

The technique used was as follows: The bacterial suspensions, from 18-hour cultures on agar slants, were made in saline containing 0.1% gelatin. Very few bacteria survived incubation in saline, but the addition of this very small amount of gelatin removed the toxicity of the saline. The first dilution was made to correspond with Tube 1 of the McFarland nephelometer, the final dilution was one ten-thousandth of the first. The number of bacteria on control plates without serum was 1,000 to 2,000 per cc., 1 cc. being the amount of suspension used in the tests. The mixtures of bacteria and serum were incubated for 4 hours in a waterbath at 37.5°C., and the entire mixture was plated by pouring. Complete inhibition

[1] Bordet, J., *Traité de l'immunité,* 1920, 320.

[2] Bordet, J., *Traité de l'immunité,* 1920, 95.

[3] Muir, R., and Browning, C. H., *J. Path. and Bact.,* 1909, **13**, 76.

[4] Gordon, J., and Wormall, A., *J. Path. and Bact.,* 1928, **31**[2], 753.

TABLE I.
Bactericidal Action of Normal Human and Rabbit Serum.

	Human		Rabbit		
	Active	Inactivated 56° C.	Active	Inactivated 56° C.	Controls, no serum
			{+++	++++	
B. typhosus Rawlins	—	++++	{—	++++	++++
" F	—	++++	++	++++	++++
B. dysenteriae Flex 2	++	++++	++	++++	++++
Shiga 1			+	++++	++++
Shiga 2	—	++++	+	++++	++++
B. coli 1	—	++++			++++
B. aerogenes			—	++++	++++
B. paratyphosus A	—	++++	++	++++	++++
B	—	++++	—	++++	++++
Proteus X 19	—	+++	++	++++	++++
			++++	++++	
" from rabbit	++++	++++	++++	++++	++++
B. alkaligenes	++	++++	++++	++++	++++
B. morgani	—	++++	++++	++++	++++
B. suipestifer	—	++++	—	++++	++++
B. icteroides	—	++++	—	++++	++++
B. aertrycke (mouse)	—	++++	++++	++++	++++
B. enteritidis (mouse)	+++	++++	++++	++++	++++
" (old laboratory str.)	—	++++	{—	++++	++++
			{++++	++++	++++
B. pyocyaneus	—	++++	++	++++	++++
B. bronchisepticus			++++	++++	++++
B. prodigiosus	++++	++++	++++	++++	++++
B. subtilis (old lab. str.)	++++	++++	++++	++++	++++
" (Soule)	+	++++	—	—	++++
B. anthracis	+++	+	—	—	+++
B. friedländeri (old lab. str.)			++++	++++	++++
(non-mucoid)	++	++++	+	++++	++++
(mucoid)	+	++++	—	++++	++++
Staphylococcus aureus (old lab. str.)	++++	++++	++++	++++	++++
" " (empyema)	++++	++++	++++	++++	++++
" " (osteomyelitis)	++++	++++			++++
" " (Brodie's absc.)	++++	++++			++++
" " (ear abscess)	++++	++++			++++
" " (nose-throat)	++++	++++			++++
Streptococcus 20	++++	++++	++++	++++	++++
M. tetragenus	++	++	+	+++	++
Sarcina lutea	—	++	—	+	++
B. diphtheriae	+++	+++			+++
B. abortus	++++	++++	++++	++++	++++
B. melitensis	++++	++++	++++	++++	++++
Pneumococcus	++	++	++	++	++

of growth is recorded as —, a few colonies as +, 50-100 colonies as ++, and abundant growth as +++ or ++++. Titrations of hemolytic complement were carried out by the antisheep system.

The complement (hemolytic) titer of the human serum, which was a mixture of specimens taken for Wassermann tests, was remarkably constant (0.02-0.05 cc.), that of rabbit serum was much lower and varied in different animals from 0.14 to 0.20. The bac-

tericidal titer in each instance was much higher than the hemolytic, being 0.001-0.0005 cc. for the human and 0.01-0.005 cc. for the rabbit. Comparative tests of several rabbit sera of high and low hemolytic titer showed no correlation between bacteriolytic and hemolytic titer.

When dog serum, which had a constant hemolytic titer of 0.05 cc., was tested for bactericidal action, it was found that only 3 bacteria in the entire collection (*B. morgani, Proteus X 19,* and *Sarcina lutea*) were killed by 0.1 cc. of serum. An experiment to test the feasibility of reinforcing the complement content of diluted or heated serum by means of dog serum revealed the fact that the dog serum was antibactericidal when mixed with fresh rabbit or human serum, a property which was not removed by heating. For example, a serum which alone was bactericidal in 0.0005 cc. was no longer bactericidal below 0.05 cc. in the presence of 0.1 cc. of dog serum (2 hemolytic units), though such a mixture showed complete lysis in the hemolytic test. Hence, although dog serum can function as complement in the Wassermann test, as shown by Noguchi and Bronfenbrenner[5] and confirmed in the present work, it not only fails to function as bacteriolytic complement but seriously interferes with bactericidal action. Bacteria submitted to bactericidal tests after treatment with dog serum proved to be only slightly more susceptible to bacteriolysis than bacteria not so treated.

The susceptibility or resistance of the various bacteria, while showing little or no variation when tested against a given animal serum, seems to follow no general rule. In some instances (*B. enteritidis, B. typhosus*) the pathogenic organisms resist bactericidal action, the non-pathogenic not, but in the case of *B. friedländeri* this finding is reversed. Some saprophytes are killed (*Sarcina lutea, B. aerogenes*), others are resistant (*B. prodigiosus*). That the mechanism is different for Gram negative bacteria[6] is not borne out, *Sarcina lutea* being killed under the same conditions as the Gram negative bacteria, instead of falling into the class with *B. subtilis* or *Staphylococcus.*

The failure of dog serum to reactivate heated human serum seems to indicate that hemolytic complement is in this case distinct from bacteriolytic complement, but no explanation offers itself for the interference of dog serum with the bactericidal action of other sera.

[5] Noguchi, H., and Bronfenbrenner, J., *J. Exp. Med.,* 1911, **13**, 78.

[6] Mackie, T. J., and Finkelstein, M. H., *J. Hyg.,* 1932, **32**, 1.

7641 C

Influence of Calcium Ions upon Energy Metabolism of the Mammalian Heart.*

HOWARD C. PETERS, CHARLES E. REA AND MAURICE B. VISSCHER.

From the Department of Physiology, University of Illinois, Chicago.

Certain of the cardiac actions of calcium are well known. Numerous experiments have demonstrated an increase in the mechanical energy liberated by the heart under the influence of calcium. The literature on the effects of this ion upon the heart has been reviewed recently by Berliner.[1] We have studied the changes in energy liberation and distribution which permit, at a given diastolic volume, an increased work output of the heart under these conditions.

The methods we have employed permit measurement of the oxygen consumption of the heart and eliminate possibility of error due to changes in the elasticity of the lungs inherent in the earlier methods.[2] The arrangement of apparatus (Fig. 1) is essentially that of

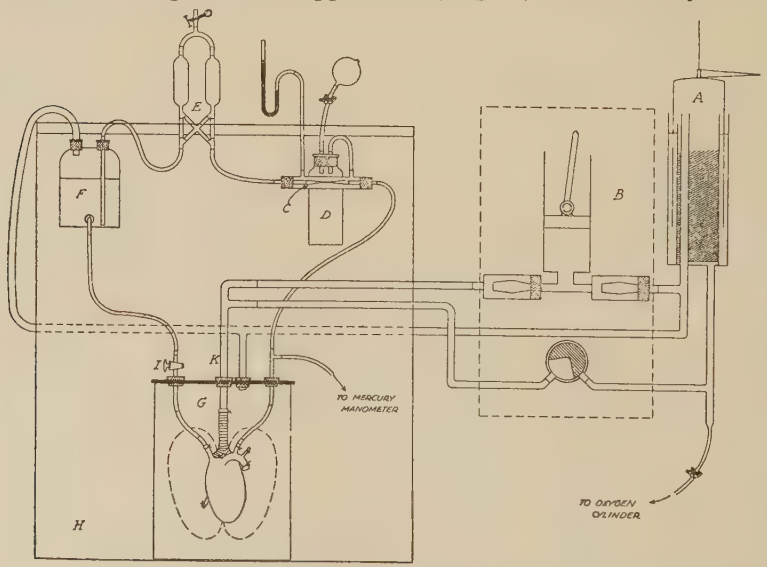


FIG. 1.

A—oxygen spirometer, B—respiration pump, C—artificial resistance, D—pressure bottle, E—stromuhr, F—venous reservoir, G—glass jar, actually horizontal, H—constant temperature bath, I—stopcock for adjustment of venous return, K—tracheal cannula.

* Aided by grant 282 from the Committee on Scientific Research of the American Medical Association.

[1] Berliner, *Am. Heart J.*, 1933, **8**, 548.

[2] Starling and Visscher, *J. Physiol.*, 1927, **62**, 243.

the usual heart-lung preparation in an entirely closed system. After the artificial circulation has been established, the heart and lungs are freed from the body of the dog and encased in an airtight glass jar, which is then connected with large rubber tubing to the oxygen spirometer and the venous reservoir, and lowered into a constant temperature bath adjusted to 37.5°. Under these conditions there is a continuity of the entire air content of the system with automatic compensation for changes in the volume of the heart and lungs. When the ventricular volume was recorded, a cardiometer, clamped rigidly in place, and communicating with a large tambour was also included in the glass chamber.

After a control period of approximately an hour calcium chloride or calcium gluconate was introduced into the venous reservoir. In the first 3 experiments of Table Ia, the experiments of Table Ib, and the first 2 experiments under "d" 1 cc. of 20% calcium chloride was injected. In experiments marked "c" ½ cc. of 20% calcium chloride was used. In the third experiment of the "d" group 1.5 cc. of 20% calcium gluconate (calglucon, Sandoz)† was given. In the fourth experiment of the "a" group and in the experiments of Table II 2 cc. of 20% calcium gluconate was injected. The blood volume of the system varied between the approximate limits, 900 cc. to 1200 cc. Oxygen consumption, arterial pressure, and, in some experiments, heart volume were recorded continuously. Volume out-

TABLE I.
Effect of calcium on energy exchanges in the heart.

Exp.	Oxygen cons. cal./min.		Volume output cc./min.		Efficiency %		Inc. efficiency %	
	Control	After Ca	Control	After Ca	Control	After Ca		
3/27/33	39.4	40.6	468	500	3.84	4.11	7.0	(a)
3/28/33	58.6	57.0	385	385	2.15	2.21	2.8	(a)
3/29/33	60.1	55.9	326	326	2.19	2.35	7.3	(a)
2/16/34	81.6	79.6	382	400	1.29	1.41	9.3	(a)
1/19/33	71.9	71.9	400	732	2.09	4.07	95.	(b)
1/27/33	46.6	43.9	366	385	2.89	3.25	12.	(b)
1/28/33	54.1	54.9	357	577	2.33	3.82	64.	(b)
2/21/33	83.4	83.4	366	484	1.62	2.24	38.	(b)
6/1/33	48.7	43.1	353	423	2.62	3.62	38.	(c)
6/3/33	36.6	46.7	333	739	2.81	5.71	103.	(c)
2/27/33	78.1	72.6	492	541	2.30	2.77	20.	(d)
3/25/33	46.2	37.0	366	380	2.89	3.75	30.	(d)
5/4/34	74.2	78.6	462	1000	1.99	5.35	169.	(d)

(a) In the fresh heart.
(b) Following injection of parathormone.
(c) Following injection of ethyl alcohol.
(d) Spontaneously hypodynamic heart.

† We desire to thank the Sandoz Company for their courtesy in supplying us with this material.

TABLE II.

Effect of Calcium on Energy Liberation and Efficiency of Hypodynamic Heart at Approximately Constant Diastolic Volume.

Exp.	Diastolic vol.change due to Ca cc.	Oxygen cons. cal./min.		% inc. in O_2 cons. corrected	Efficiency %		% inc. efficiency corrected
		Control	After Ca		Control	After Ca	
4/6/34	— .2	32.9	45.3	47	4.52	7.97	65
4/13/34	—1.1	45.4	55.6	28	2.80	5.42	85

put and rate were determined at frequent intervals. Averages are given in the tables.

The estimated efficiencies are based on the external work of the heart only and include an error due to the metabolism of the lungs. They therefore express the external efficiency of the preparation rather than the efficiency of the heart muscle. However, since we are interested in changes in efficiency rather than absolute values, lung metabolism introduces no significant error unless the consumption of oxygen varies considerably. In the last columns of Table II this source of error is roughly corrected by subtracting 20% of the oxygen consumption value for the control period. Since calcium dilates the coronary vessels (Mancke,[3] Hochrein[4]), measurement of changes in external output will not exaggerate but rather minimize changes in the total output. The external efficiency changes observed thus seem to represent real increases in muscular efficiency.

In all our experiments, except one in which no change was observed, calcium produced a slight increase in the heart rate. The average value of this increase was about 6%.

While the increase in frequency and the tonic effect of calcium upon the heart volume are seemingly independent of the previous treatment of the heart, the changes in cardiac output and efficiency due to calcium depend on the condition of the preparation at the time of injection. Fresh preparations respond to calcium with little or no increase in cardiac output and a hardly significant increase in efficiency (Table Ia). In hypodynamic preparations, however, injection of calcium is followed by a definite increase in cardiac output and efficiency (Table Ib, c, d). The beneficial effects are of relatively long duration, although the exact length of time cannot be adequately studied in this preparation because of the continuous spontaneous dilatation. The figures recorded in the tables are based upon the initial 10 or 15 minute period of maximum change in the variables studied.

[3] Mancke, *Arch. exp. Path. Pharm.*, 1930, **149**, 67.

[4] Hochrein, *Klin. Wchnschr.*, 1931, **10**, 1705.

In the experiments of Table II the diastolic volume was maintained nearly constant by adjustment of the venous inflow. In both experiments the injection of calcium was followed by a definite rise in total energy liberation measured as oxygen consumption, as well as a marked increase in efficiency. It appears from the observations of Starling and Visscher[2] that the increase in rate cannot account for more than a 10% increase in oxygen consumption in these experiments. There is therefore a definite increase in oxygen usage which is independent of the rate.

Summary. At a given initial fiber length the mechanical energy of contraction of the heart is increased under the influence of calcium. This is due not only to an increase in the fraction of the total energy liberated which is converted into useful work but also to an increased total energy liberation. The increase in efficiency occurring with administration of small doses of calcium salts indicates that the effect is a beneficial one, and might be of importance in contributing to the therapeutic effect of calcium in cardiac disease.

7642 P

Bromide Control of Experimental Convulsions.

BENJAMIN BOSHES. (Introduced by L. J. Pollock.)

From the Department of Nervous and Mental Diseases, Northwestern University Medical School.

Since Locock[1] reported the successful use of bromides in several cases of epilepsy, this drug has been used extensively in the treatment of this disease. When these patients have been so treated, it has been noted that the concentration of the bromide ion in the blood is not always related to the amount of the drug administered; or is this concentration parallel to the clinical course, either in regard to the control of the convulsions or to the development of bromide intoxication.

Since the amount of bromide in the blood necessary to prevent epileptic convulsions in man varies, a determination of the concentration necessary to prevent experimentally produced convulsions in rabbits was made. The convulsions were induced by the intravenous injection of 2% thujone in 6% gum acacia, using 0.15 cc. per pound body weight. Seizures always followed such a dose, providing the

[1] Locock, Sir C., *London Lancet*, 1857, **1**, 528.

TABLE I.
Relation of Blood Bromide Levels to Thujone Convulsions.

No.	Date	Total Halide as mg. of NaCl per 100 cc. of blood	Bromide as mg. of NaBr per 100 cc. of blood	Convulsion
64	12-13	466	0	Moderate
	12-22	473	60	Severe
	12-27	455	150	None
	12-30	460	140	"
63	12-30	476	90	Moderate
29	10-12	480	140	Severe
12	12-9	468	225	" but brief
31	10-16	475	240	None
3	7-19	446	300*	"

*38% chloride replacement. This was calculated by multiplying the bromide concentation by 0.568 (the factor necessary to convert bromide values to equivalent chloride values) and dividing the figure so obtained by the total halide concentration considered as NaCl.

injections were not repeated within one week. Rabbits were fed 5 grains of sodium bromide daily by stomach tube. When a blood bromide level of 240 mg. per 100 cc. of blood as measured by the Wuth[2] method was reached, injection of the thujone no longer produced a convulsion. In some animals a lower concentration sufficed to prevent induced seizures. (Table I.)

The total blood halide remained unchanged even when there was a 38% replacement (Table I) of the chlorides by the bromide; one may readily conclude that bromide replaces chloride, ion for ion.

To determine the site of action of the bromide, the amount in the brain was compared to that in the blood. The Berglund[3] method of analysis was used. The chloride content of the brain varied in rabbits, but the average was 166 mg. per 100 gm. of brain. The brain bromide content in animals fed 5 grains of sodium bromide over different periods of time varied from 7.5 to 95 mg. per 100 gm. of brain while the respective blood bromide ranged from 40 to 300

TABLE II.
Comparative Bromide-Chloride Levels in Brain and Blood.

No.	Blood Halide as mg. NaCl per 100 cc. blood	Blood Bromide as mg. NaBr per 100 cc. blood	Brain Halide as mg. NaCl per 100 gm. brain	Brain Bromide as mg. NaBr per 100 gm. brain	Convulsion	Blood Chloride replacement %	Brain Chloride replacement %	Ratio Brain Bromide to Blood Bromide
8	390	136	167	50	Moderate	19.8	17	1:2.7
12	431	206	112	14	Strong	27.1	7.1	1:14.7
13	393	46.4	156	7.5	Mild	6.7	2.7	1:6.2
19	427	193	183	53	"	25.7	16.4	1:3.6

[2] Wuth, O., *J. Am. Med. Assn.*, 1927, **88**, 2013.

[3] Berglund, Emil, *Z. f. Analytische Chem.*, 1885, **24**, 184.

mg. per 100 cc. of blood. (Table II.) There was no definite relationship between the bromide concentration in the brain and that of the blood. The ratio of brain bromide to brain chloride bore no constant relation to the ratio of blood bromide to blood chloride. The bromide content of the brain seemed closely related to the amount of blood left in the brain after the heparinized animal had been bled from the carotid arteries. The presence of so little bromide in a well exsanguinated brain, when there was a high concentration of bromide in the blood, suggested that the action of bromide was effected in some manner other than by combining with nervous tissue as postulated by Bernoulli.[4]

The question of whether the bromide acted by lowering the chloride level of the body was next studied. Attempts to lower the blood chloride by feeding large doses of potassium citrate as suggested by von Bunge[5] were unsuccessful. Perfusion of the peritoneal cavity with warm distilled water produced a marked hypochloremia (Curtis[6]) in rabbits. Such animals were even more susceptible to the convulsant, usually dying in *status epilepticus*. Finally, a study was made to determine whether the chloride ion increased the susceptibility to convulsions. Large doses of sodium chloride were fed to bromized animals, and normal rabbits were given combined sodium bromide and sodium chloride feedings. In both instances, the studies indicated that the chloride acted by diminishing the bromide

TABLE III.
Effect of Sodium Chloride on Thujone Convulsions in Bromide-Fed Rabbits.

No.	Date	Fed	Total Halide as mg. NaCl per 100 cc. blood	Blood Bromide as mg. NaBr per 100 cc. blood	Convulsions
61	4-1	(Start of experiment)	460	0	No attempt made to induce convulsion
	4-1 to 4-11	NaBr, NaCl, aa gr. V			
	4-11		430	90	Severe
	4-11 to 4-20	No feeding of NaCl or NaBr			
	4-20 to 4-28	NaBr gr. V daily			
	4-28		442	105	Moderate
	4-28 to 5-5	NaBr gr. V daily			
	5-5		475	165	No attempt made to induce convulsion
	5-6	NaCl gr. XXX			
	5-7	No feeding	428	115	None

[4] Bernoulli, E., *Arch. f. exp. Path. u. Pharm.*, 1913, **73**, 355.

[5] von Bunge, G., *Lehrbuch der Physiologie des Menschen*, 1901, **2**, 103.

[6] Curtis, G. M., *Proc. Inst. Med. Chicago*, 1930, **8**, 110.

content of the blood to a concentration where convulsions could be produced experimentally, if no additional chloride was administered. (Table III.)

7643 C

Effect of Heavy Administration of Viosterol on Metabolism of the Rat.

C. I. REED.

From the Department of Physiology, University of Illinois College of Medicine.

In a previous communication[1] it was shown that when large doses of irradiated ergosterol were administered to normal dogs there was a very great increase in metabolic rate. Since it appears that the rat is relatively insensitive to toxic effects of this substance, it seemed advisable to investigate its effect on the metabolism of the rat. Three rats of each sex were selected and trained for several months in a metabolism chamber. They were kept in separate cages and fed on the Wistar normal diet. The cages were kept in the same room where the apparatus was operated during a period of nearly 10 months, and the rats were handled only by the operator. It is believed, therefore, that extraneous factors were eliminated as completely as possible.

The apparatus used consisted of a closed circuit with a motor-driven hydraulic pump that delivered approximately 90 cc. of air each half minute. The respiration chamber consisted of a glass jar. Volume change was recorded on a kymograph by means of a writing needle attached to the bell of a small spirometer cut into the circuit. There was no new principle introduced into the arrangement.

The surface area was calculated by the formula derived by Lee,[2] $S = KW^{0.60}$ in which $K = 12.54$, $W =$ weight in gm., and $S =$ surface area in cm^2. It was mentioned in the earlier publication that one of the difficulties in calculating the surface area in the dog was the varying weight. Since the rat weight also varied it was thought necessary at first to use Lee's modification of Cowgill's formula,[3] $S = KW^{0.61} \times \frac{0.31}{Nobs}$ but comparisons of surface area calcu-

[1] Reed, C. I., Thacker, E. A., Dillman, L. M., and Welch, J. W., *J. Nutrition*, 1933, **6**, 355.

[2] Lee, M. O., *Am. J. Physiol.*, 1929, **89**, 24.

[3] Cowgill, G. H., and Drabkin, D. I., *Am. J. Physiol.*, 1927, **81**, 36.

lated by the 2 formulae on the same animal made it apparent that for purposes of comparison over a period of time there was not enough difference to justify the use of the more complicated formula.

The irradiated ergosterol was in the form of viosterol 10,000X,* which contains 920,000 International Units per cc. Where dilution was necessary a highly purified grade of corn oil was used. The material was administered by mouth by means of a small bore silver tube attached to a hypodermic needle. This, in turn, was fitted onto a 1 cc. tuberculin syringe. In this way accurate administration was insured.

F.1. The average metabolic rate from March 17 to June 6 was 801 ± 39 cal/M²/24 hrs. During this time the weight increased from 166 gm. to 180 gm. Beginning on June 6 a dose of 0.1 cc. of 10,000X viosterol (92,000 I. U.) was administered daily for 6 days. During this time the average rate was 1127 cal/M²/24 hrs., but on June 14 it fell to 676 cal. and continued definitely low but very irregular to August 1, when the original level was attained except for 2 high points on June 27 and July 10. The average for this period was 740 cal.

The weight decreased from 180 gm. to 146 gm. on June 20, then began to increase rather rapidly until on July 3 when the animal weighed 171 gm. During the next month the gain was less rapid. The original weight of 180 gm. was not regained until August 1.

On August 16 was begun the daily administration of 9200 I. U. of viosterol over a period of 12 days, during which time the average rate was 796 ± 15 cal/M²/24. On August 28 the daily dose was increased to 20,000 I. U. for 4 days. There was a progressive increase in the rate to 973 cal. on September 6, after which there was a progressive decline to the end of September to the original level. The weight fluctuated some but the average for the entire period up to September 15 when began a steady increase, was 176 gm.

F.2. Average metabolic rate from April 1 to June 6, 830 ± 23 cal/M²/24 hrs. On the latter date the weight was 185 gm. June 6 to 10 inclusive 0.2 cc. daily of 10,000X viosterol were administered (184,000 I. U.). There was no pronounced alteration until June 14, when the rate suddenly fell to 408 cal/M²/24 hrs. The animal died 6 hours later. The weight had decreased to 142 gm.

F.3. Average rate April 1 to June 6, 834 ± 50 cal/M²/24 hrs. Weight on June 6, 154 gm.

Three daily doses of 0.5 cc. 10,000X viosterol (460,000 I. U.)

* Supplied by Mead Johnson & Company.

were administered beginning on June 6. The weight declined progressively to 126 gm. on June 13. The rate dropped sharply to 700 cal. on June 9, then increased to 907 on June 12. The animal died the following day.

M.1. Average rate, April 1 to June 1, 754 ± 31 cal/M^2/24 hrs. Weight on June 6, 252 gm. At this time 0.2 cc. daily of viosterol 10,000X (180,000 I. U.) were administered over 3 days. The weight declined to 214 gm. on June 14, but was restored to 252 gm. on June 21. The animal continued to gain slowly to 264 gm. on August 16, when the second period of administration was begun. This comprised 30,000 I. U. daily over 4 days.

On June 7 the rate was 880 cal/M^2/24 hrs., thereafter declining progressively to 580 cal. on June 14 and remaining in this level until June 21 when there was a second increase finally mounting to 1140 cal. on June 27. The average for the second period of increased rate was 958 ± 182. Up to July 25 there was a second low period averaging 614 ± 106. Thereafter the original rate was restored.

With the second period of administration the rate increased promptly and progressively to 1010 cal. on August 21 and reached the original level on September 5. The average during this period was 913 ± 103 cal.

M.2. Average rate, April 1 to June 1, 735 ± 41 cal/M^2/24 hrs. Weight June 6, 306 gm. June 6 to 8 inclusive 0.6 cc. viosterol 10,000X (552,000 I. U.) daily. The rate declined from 753 cal/M^2/24 hrs. on June 6 to 582 on June 12. The weight declined during this period to 248 gm. This animal was found dead the following day.

M.3. Average rate during the entire period from April 1 to September 1 was 780 ± 63 cal/M^2/24 hrs. Weight at the termination of the experiment, 244 gm. This animal was retained as a control.

From these experiments it is apparent that the basal metabolic rate of the albino rat may be increased markedly if the dose administered is not too large. Even with a toxic dose there is a transient elevation of the rate followed by a decline below the original level. Three of the 5 animals died. The 2 survivors continued to exhibit an irregular but low average rate for several weeks, followed by a normal period.

This extreme irregularity was displayed by the dogs also, but there was little tendency to depression below the original level. Possibly the inherent errors in calculation of the rate account for the difference but this seems unlikely.

It appears then that vitamin D in the form of viosterol produces an increase in the metabolic rate in the normal rat similar to that previously reported for normal dogs.

7644 P

Acid and Enzyme Content of Postoperative Emesis as Indication of Regurgitation from the Duodenum.

EDMUND ANDREWS AND PAUL H. HARMON.

From the Department of Surgery, the University of Chicago.

The lack of knowledge concerning the cause of the nausea and vomiting which follows surgical operation prompted these studies. We have examined 115 specimens of postoperative vomitus from 62 cases chosen at random to include anesthetics and operations of many types. Over half had had ether either alone or in combination with other anesthetics. These factors seemed to bear no relationship to the results tabulated below.

No free acid	95	specimens
0 to 20° free acid	9	"
20 to 40° free acid	4	"
40 to 60° free acid	4	"
80° or more free acid	3	"
Total	115	"

Cushing[1] has recently called attention to a possible nervous mechanism in the production of peptic ulcer. In this connection it is interesting to note that of 11 specimens with an acidity over 20, 7 were obtained following operations for brain tumors. This observation might be explained by Cushing's postulation of a center in the cerebrum which is capable of promoting gastric secretion.

Not only the cases that actually vomit have this anacidity. Postoperative aspiration was done by nasal catheter in 15 patients. In these specimens there was no free acid. This lack of acid is not accompanied by a corresponding lack of enzymes. In all cases where free acid was present peptic activity was present, and in most of the cases (91 of 115) where the acid was absent peptic activity was detected after acidification with 0.4% hydrochloric acid. Rennin was present in 80 of 115 specimens.

[1] Cushing, H. K., *Surg. Gynec. and Obst.*, 1932, **55**, 1.

Evidence of duodenal regurgitation is present as witnessed by the fact that of 115 cases, only 57 had bile in the stomach, while a considerably greater number contained pepsin but were not acid. Specimens from 75 cases were found to contain trypsin. Twenty-five of these 75 samples contained no bile. It is apparent, therefore, that regurgitation of duodenal juices without bile is actually more common than with bile. The *a priori* assumption that the presence of bile in a specimen from the stomach is an indication of duodenal regurgitation is thus invalidated.

The well known intolerance for food of postoperative patients is therefore associated with a total achlorhydria in most cases. Our experiences indicate a common lack of free gastric acidity, which might be an obvious explanation of the clinical phenomenon. Almost uniform presence of pepsin with little or no free acid in gastric contents vomited after operations indicates that reduction in acidity is due to neutralization by duodenal contents rather than by lack of active gastric secretion. It is important to recall that pancreatic juice contains large amounts of sodium carbonate which will be liberated on acidification. An obvious mechanism is suggested for the rapid formation of gas in the stomach. If ordinary gastric juice and pancreatic juice be mixed *in vitro* the amount of gas produced is very striking as they effervesce almost like a Seidlitz powder.

Boldyreff's theory of the acid control by alkaline reflux can thus probably be widely applied. Jones[2] showed that pancreatic juice had considerable more neutralizing power than bile and it is secreted in larger amounts. Under the conditions with which these data were collected the secretion of bile is at a minimum. Whipple[3] and many others have showed the delicate mechanism of bile excretion. Ether in particular has a very marked depressing effect. Following the operative establishment of a biliary fistula, the flow is very scant for the first day or two.

The obvious deduction to be drawn from these studies is that in the treatment of postoperative vomiting and nausea, measures are indicated which would increase the gastric acidity.

---

[2] Jones, K. K., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 567.

[3] Whipple, G. H., *Physiol. Rev.*, 1922, **2**, 440.

7645 C

A Study of Muscle Temperature During Bacterial Chill.

A. J. NEDZEL.

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

It still remains the general opinion that heat production in the body depends mainly on the muscles, although it is admitted that a certain amount of heat is liberated by the chemical processes occurring in viscera, especially the liver. In comparison to the amount of heat produced by the muscles it is considered insignificant. Moreover there are different chemical processes that occur in the liver, which actually absorb heat, instead of producing it. In other words, any noticeable increase in the body temperature is attributed to the muscular activity.

Since the production of heat is constantly associated with the activity of the muscles and especially when the muscles are in action, it is also generally accepted that any tremor of muscles is accompanied with heat production. The bacterial chill, in which we have a contraction of muscular fibers and rigor, is accompanied with an elevation of body temperature. This increased heat production is usually attributed to apparent muscular activity. Briefly, to the muscular tremor, whenever it occurs, we ascribe heat production and associate the rise in temperature of the patient with bacterial chill to this cause.

In disagreement with this general opinion Petersen and Müller[1] presented experimental evidence indicating that the chill in the dog produced by continuous injection of colon bacilli is always associated with an increase in the splanchnic activity on the one hand and muscular tremor and leukopenia on the other. But this muscular activity is not accompanied by increased temperature in the muscles themselves.

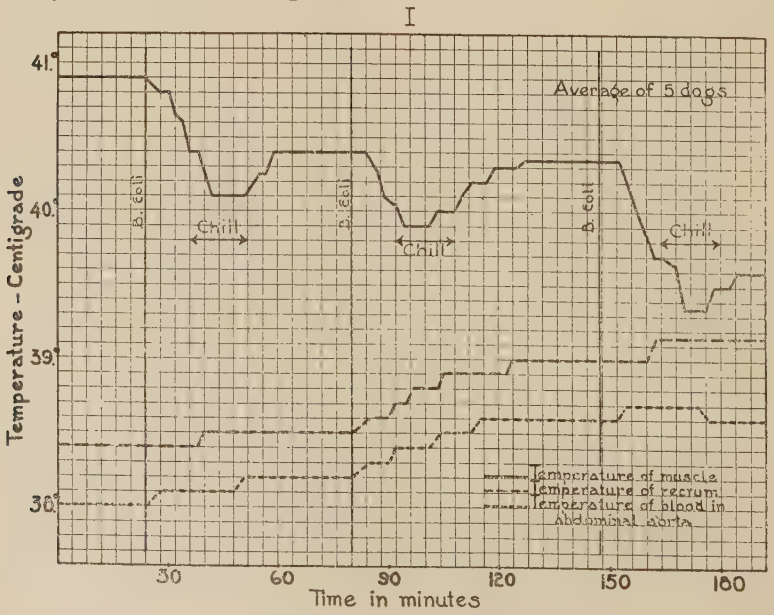
The temperature determinations were made with clinical thermometers inserted into the body cavities and into the skeletal muscles and inasmuch as the temperature changes are presumably of small degree, these determinations might be subject to criticism. They particularly stressed the importance of the splanchno peripheral balance and emphasized the general vasoconstriction of the peripheral tissues as well as the fact that the "muscle tremor" of bacterial chill is to be regarded as an expression of a wholly differ-

[1] Petersen, W. F., and Müller, E. F., *Arch. Int. Med.*, 1927, **40**, 575.

ent mechanism than that of normal muscle contraction which is of course associated with prompt vasodilatation.

In our studies of temperature changes on the surfaces of the body, and in the liver and kidney in their relation to heat and cold on the skin we introduced the simultaneous measuring of the temperature in the abdominal aorta which is more or less stable in comparison with the temperature of the body surfaces and viscera and these studies have been extended to a re-investigation of the muscle temperature in bacterial chill using a very sensitive thermocouple for the determination of the muscle temperature.

Our experiments were conducted as follows: Under nembutal anesthesia we gained access to one muscle of the dog's thigh and bloodlessly separated the fibers of it so that 3 thermocouples (devised by Dr. Bachem) could be easily introduced into the muscular tissue. Then the skin was brought together and the thermocouples were fastened with adhesive to the skin in such a manner that they were held firmly in place in the muscle tissue. Heparin was injected intravenously (0.01 per kilo), the femoral artery opened and a long thin glass thermometer introduced through it into the abdominal aorta. An ordinary glass thermometer was introduced into the rectum for about 2 or 3 inches and fastened. A suspension of half an agar slant of *B. coli* in 20 cc. of NaCl solution was injected intravenously at the time through the femoral vein.



The results are shown on the accompanying graph. The abscissa represents the time from the beginning of the experiment, the ordinates, temperature in centigrade. The straight lines represent the temperature of the muscle, the dotted line temperature of the blood in the abdominal aorta and the broken line temperature in the rectum. The perpendicular lines show the time of injection of the bacterial suspensions.

Graph represents an average of the results of experiments on 5 dogs. The temperature in the abdominal aorta and in the rectum gradually rises, while the muscle temperature falls after each injection of *B. coli* suspension.

The results, as observed in our experiments, substantiate the findings of Petersen and Müller.[1] During the visible bacterial chill there is a rise in the body temperature, as shown by temperature recordings in the abdominal aorta and rectum, but this temperature is not generated in the muscles. On the contrary, the muscular temperature falls. Of course, with time the muscle temperature rises after the readjustment of the splanchno peripheral balance, but at least not in the beginning of a visible chill.

The muscular tremor which was observed during the bacterial chill is not accompanied with an increase of the temperature in the skeletal muscles, so this muscular activity is of a different type than the one we have in ordinary muscular work, which is accompanied by production of heat.

7646 P

Temperature Changes in Gastrointestinal Tract in Relation to Heat and Cold on Skin.

A. J. NEDZEL.

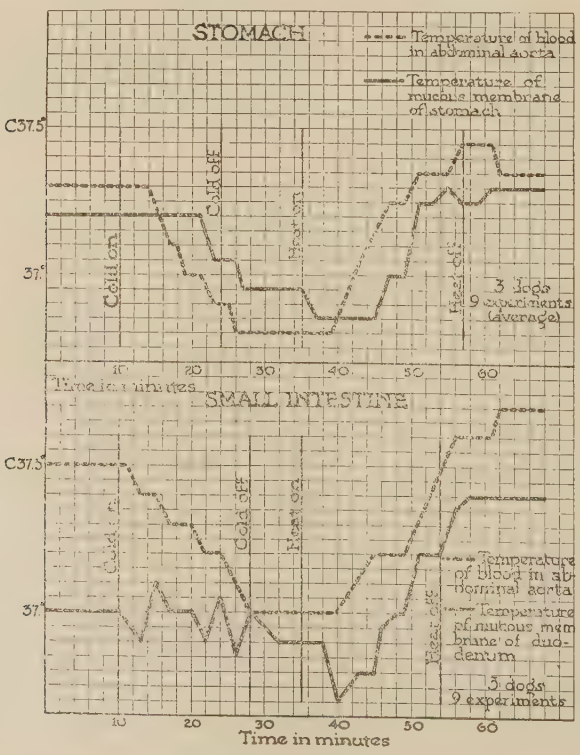
From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratories of the Illinois Department of Public Health.

For our temperature readings of a mucous membrane of gastrointestinal tract we used a Queen's potentiometer.

Ten dogs were used. Under nembutal anesthesia, the abdomen was opened and an opening in the stomach or intestine was made. For measurements of temperature of mucous membrane of the stomach the ends of 3 thermocouples (devised by Bachem), covered

with shellac and embedded in celloidin, were introduced into the stomach and fastened to the inner surface by a ligature. For recording the temperature of the mucous membrane of the intestine the 3 thermocouples mounted on a piece of a rubber tubing of suitable diameter, and fastened with a rubber band were introduced into the intestine (duodenum). For measuring the temperature of the rectum the thermocouples were introduced through the anus. Heparin was injected intravenously (10 mg. per kilo), the femoral artery opened and a long glass thermometer introduced through it into the abdominal aorta. Ice packs and hot moist towels were applied to the skin of the chest of dogs.

The results are shown on the accompanying graphs. The abscissa represents the time from the beginning of the experiment, the ordinates—temperature in centigrade. Dotted lines represent the findings of the temperature in the abdominal aorta, the straight line—the temperature of the surface of the mucous membranes. The perpendicular lines show the time of application of cold and heat to the body surface of the dog.



The application of cold or heat to the skin causes a gradual fall or rise of the temperature of the blood in the aorta. The same change is observed on the mucous membrane of the stomach with the exception that the fall of temperature here after application of cold is delayed and application of heat evokes the rise later than in the aorta.

In the mucous membrane of the duodenum and of the central part of the rectum there is also a tendency to a delayed response of the mucous membrane in fall or rise of its temperature on the application of cold or heat to the skin. This decline and elevation of temperature is irregular, that is in a wavelike manner.

The temperature of the mucous membrane of rectum near the anus shows a delayed response in the lowering of its temperature after application of cold to the skin (in comparison with the temperature in aorta), but in addition it also shows a difference in recovery after the application of heat. The recovery is delayed.

Pacific Coast Section.

Stanford University, October 20, 1934.

7647 P

Brucella Phagocytic Index Test.

K. F. MEYER, B. STEWART, L. VEAZIE AND B. EDDIE.

From the George Williams Hooper Foundation, University of California, San Francisco.

Epidemiologists have observed that (a) a percentage of persons exposed to the risk of infection with Brucella-type abortus or suis-show either agglutinins or complement fixing antibodies in their serum and (b) clinical undulant fever is relatively infrequent in the occupational groups who come continuously and intimately in contact with Brucella organisms. Various inquiries leave no other explanation than that the *Brucella abortus* and probably the *Brucella suis* and *melitensis* as well are organisms endowed with a moderate degree of virulence, which rather frequently infect persons who are exposed either by contact through the skin or by alimentary ingestion. These infections are quite often subclinical and remain latent. Surveys in occupational groups have shown that the serologic tests fail to furnish a true picture of the number of unrecognized infections and therefore the allergic skin tests have been employed. Although a much more sensitive reagent to detect the existence of latent infections, the skin allergy is a variable state and in part influenced by the occupational activity. With the aid of this test one may recognize the allergic but not necessarily the latent infected and immune human being. The observations made by Huddleson, Johnson and Hamann[1] that the polymorphonuclear cells of persons who have had undulant fever in the past years or are actively infected or have no definite history of the disease may show a strikingly increased phagocytic activity offers a third procedure to detect latent infections. With the aid of a slightly modified *Veitch technique*[2] the usefulness of the method has been verified.

[1] Huddleson, Johnson and Hamann, *Am. J. Pub. Health*, 1933, **23**, 917.

[2] *J. Path. and Bact.*, 1908, **12**, 353.

TABLE I.

Group	No. in group	No. positive	% positive	No. negative	% negative	Aver. index	Range of indices	Remarks
Control groups								
Medical students	94	6	6.3	88	93.7	.74	0 to 18.8	All positives had definite history of contact
Dental "	27	0	0	27	100	.28	0 " 0.68	
Clinic patients	67	3	4.4	64	95.6	.49	0 " 10.84	
Patients with other febrile diseases	13	3	23	10	77	1.68	0 " 10.04	2 tularemia patients 2.4 and 10.04
Miscellaneous	24	0	0	24	100	.4	.08 " 1.08	
	225	12	5.2	213	94.8	.6	0 " 18.8	
Undulant fever patients	22	21	95.4	1	94.6	10.44	0.72 " 25.76	
Recovered cases	4	4	100.0	0	0	13.51	7.04 " 22.69	
Exposed groups								
Meat packers	388	221	57.4	167	42.6	4.07	0 " 31.2	
I. Butchers, etc., directly handling animals	161	108	67	53	33			Average index of 221 positive meat packers, 6.64
II. Skins, wool, etc.	111	55	49	56	51			
III. Truck drivers, feeders, office force	116	58	50	58	50			
Veterinarians	102	74	73	28	27	4.95	0 " 21.16	
Laboratory workers	17	17	100	0	0	13.6	3.36 " 28.4	Only those working with Brucellas included

One or 2 drops of 1.6% sodium citrate solution are dried in a small tube. At the time of the test 1 or 2 drops of human blood, either obtained from a skin puncture or by syringe, are placed in the tube. To the fluid blood are added within 30-45 minutes 1 to 2 parts of a suspension of Brucella organisms (48 hours old culture in buffered salt solution with a density of Gates 2; suis type culture No. 80 is particularly suitable). The blood-bacterial suspension is gently shaken and incubated in a water bath for 30 minutes at 37°C. Smears are then made from the sediment, air dried, fixed with methyl alcohol and stained with polychrome methylene blue. The number of ingested bacteria in 25 to 50 granulocytes is counted and the average estimated.

The data presented in Table I indicate that persons who never had contact with Brucella organisms furnish a phagocytic index below 1, the clinic patients, the medical and dental students, with few exceptions, belong in this group. Those who had leucocytes with an increased phagocytic activity came from rural communities and their histories suggested an exposure to the Brucellas in the past. By comparison the high indices from 1 to 28.4 in groups of clinical undulant or recovered cases, the laboratory workers and veterinarians strongly suggest that the enhanced specific phagocytic activity is the sequel of a recognized or latent infection. The same deductions are probably applicable to the majority of indices determined on the employees of several meat packing plants. The opsonophagic power of whole blood expressed as an index is simple and the method lends itself to epidemiologic and clinical studies. Although some persons with an index below 1.0 have developed undulant fever since the examinations had been made, it appears premature to conclude unconditionally that one phagocytic index test may decide the susceptibility or resistance to a Brucella infection. In the course of several surveys of groups exposed to the hazard of occupational undulant fever persons were found who gave a strong allergic skin test, a definite agglutination or complement fixation reaction (1:40, 4+) with a phagocytic index below 1.0. Neither their past nor present history suggests that these employees are infected or susceptible.

7648 P

Action of Stains on Living Bacteria.

F. R. SMITH AND C. S. MUDGE.

From the Division of Dairy Industry, College of Agriculture, University of California.

In studies on the streptococci and lactobacilli now in progress it became necessary to carefully examine the methods used in staining bacteria. The staining technique of the bacteriologist, for the most part, has advanced very little beyond that employed by the early workers. Perhaps Dobell[1] did more to point out certain fallacies of these methods than any other, yet his work is rarely quoted. The seemingly accepted technique is to dry on a slide the film of liquid containing the organism. After flaming, aqueous solutions of dye are flooded over the films. The delicate cytoplasm of the cell must necessarily be somewhat distorted by such methods. There are some descriptions of vital staining but generally little has been accomplished. We attempted to study the subject, using, as suggested by Dobell, a very large cell. In this case a Gram-positive organism from the gut of a guinea pig was used.

The method of staining was to add a water solution of the stain directly to either a broth or saline suspension of the organisms. The stained bacteria were examined in both wet and dry preparations. Certain stains revealed granules of varying arrangement and size within the cell. From the work of Guillermond[2] these were in all probability metachromatic granules. (Table I.) Dobell's results would agree since he says, "In my experience, only non-living structures in the cells (metachromatic granules, etc.) can be stained during life. But doubtless much depends on the stain itself!"

Gay and Clark[3] suggested that "vital" staining may really only represent a stage in a process of injury by the stain. In an attempt to test the possibility of injury to the cell experiments were designed to determine the toxicity of the various stains. The method employed generally for this test was to mount the suspension of organisms under a cover-slip and allow the stain to diffuse through from one side. This permitted the worker to follow the effect of the stain on the organism. The majority of the basic stains under examination were found to be toxic for the particular bacterium

[1] Dobell, C. Clifford, *Quart. J. Micro. Sci.*, 1910-11, **56**, 396.

[2] Guillermond, A., *Bull. Inst. Pasteur*, 1906, **4**, 145.

[3] Gay, F. P., and Clark, A. R., *J. Bact.*, 1934, **27**, 175.

under observation. (Table I.) Methylene blue was apparently least toxic of the basic stains. Acid dyes proved to be non-toxic and also failed to stain the organism.

TABLE I.

Stain	pH	Intensity of Stain	Metachromatic Granules	Toxicity of Stain
Thionin	6.6	3+	—	2+
Malachite Green	"	3+	—	4+
Methylene Blue	"	3+	+	+
Bismarck Brown	"	2+	—	3+
Neutral Red	"	2+	+	+±
Nile Blue Sulfate	"	3+	2+	3+
Brilliant Green	"	2+	±	3+
Basic Fuchsin	"	3+	—	2+
Diazine Green	"	3+	—	2+
Safranin O	"	2+	—	2+
Rose Bengal	"	2+	—	2+
Gentian Violet	"	4+	—	3+
Orange G	"	—	—	—
Acid Fuchsin	"	—	—	—
Eosin B	"	—	—	—
" Y	"	—	—	—
Congo Red	"	—	—	—

Churchman[4] was able to stain *S. marcescens* with gentian violet and still preserve motility. We were unable to find motility when either marcescens or the Gram-positive organism was stained with gentian violet. When methylene blue was used it was possible to find a few organisms (both Gram-negative and positive) which were definitely stained and motile. This motility soon ceased, usually lasting only a minute or two after staining took place. There were, however, two variables which must be considered (1) time and (2) concentration of the stain. Thus if one were able to carefully regulate the stain to the desired concentration it is possible the time might be increased. There was no evidence that the Gram-negative organism remained motile longer after staining than the larger Gram-positive one. It did, however, remain unstained in a concentration of the dye which stained the positive organism and caused the cessation of motility. There would seem to be every reason to believe that "vital" staining was not obtained with any of the above stains, since in every case there was definite evidence of injury to the cell.

In all probability there is no direct relationship between loss of motility and death of the organism. To test this point the organisms were exposed to the stain until they were completely stained and had lost their motility. They were then centrifuged out and washed

[4] Churchman, J. W., *J. Exp. Med.*, 1912, **16**, 221.

with sterile distilled water. After all the excess stain had been removed they were centrifuged down once more and a large number added to broth. One was then able to watch the recovery of the organisms under the microscope. It was found that the bacteria gradually lost their stain, then after an interval of about 10 hours most of them had again regained their motility.

7649 C

Acute Toxicity of Ethyl Chaulmoograte.*

GEORGE A. EMERSON AND HAMILTON H. ANDERSON.

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

At the suggestion of Dr. H. W. Wade we studied the toxicity in rats of single subcutaneous doses of a number of representative types of ethyl esters of the fatty acids of chaulmoogra oil. Preparations included were: (1) a purified ethyl chaulmoograte supplied by Dr. R. Wrenshall, fractionated by redistillation *in vacuo* at 10 mm. Hg., containing a small amount of ethyl hydnocarpate as indicated by the iodine number; (2) mixed ethyl esters of fatty acids of *Hydnocarpus wightiana* oil prepared at Culion, containing more than 80% ethyl chaulmoograte as indicated by polariscopic measurements; (3) a similar preparation containing 0.5% iodine, prepared by Cole's method[1]; (4) crude ethyl chaulmoograte containing 4% creosote, a preparation used in India, supplied by Dr. E. Muir from clinical supplies at Calcutta; and (5) "Chaulmestrol" (Winthrop), a proprietary preparation of mixed ethyl esters of fatty acids of chaulmoogra oil. The pertinent findings summarized in Table I agree with Martins' report[2] of 40 cc./kg. as being lethal for *Leptodactylus ocelatus.*

Examination of the tabulated data indicate that: (1) purified ethyl chaulmoograte preparations are more toxic than the ethyl esters of the total fatty acids of chaulmoogra oil, presumably because of

* Part of a cooperative study of the chemotherapy of leprosy conducted by the Pacific Institute of Tropical Medicine within the Hooper Foundation for Medical Research, and the Pharmacological Laboratory of the University of California Medical School, San Francisco.

1 Cole, H. I., *Philipp. J. Sci.*, 1929, **40**, 503.

2 Martins, T., *Compt. rend. Soc. Biol.*, 1927, **96**, 474.

TABLE I.
Acute Subcutaneous Toxicity of Ethyl Chaulmoograte in Rats.

Substance	Iodine No.	Tolerated Dosage cc./Kg.	Mortality Ratio	Lethal Dosage cc./Kg.	Mortality Ratio	Time of Death	Cause of Death	Observations on Dying Animals
Ethyl chaulmoograte redistilled at 10 mm. Hg.	94.6	30	0/10	35-40	8/10	4 to 24 hr.	Pulmonary and gastro-enteric congestion	Irregular respiratory rate, followed by depression and opisthotonus before death in some animals
Crude ethyl chaulmoograte from Philippine Islands	87.6	35	0/10	40-50	5/15	12 hr. to 18 days	Pulmonary congestion	Symptoms less severe than with purified ethyl chaulmoograte
Crude ethyl chaulmoograte with ½% iodine from Philippine Islands	—	35	0/10	40-50	5/15	12 hr. to 14 days	Pulmonary congestion	Symptoms less severe than with purified ethyl chaulmoograte
"Chaulmestrol" (Winthrop Chemical Co.)	96.3	30	0/10	35-40	8/10	12-14 hr.	Pulmonary congestion	Symptoms less severe than with purified ethyl chaulmoograte
Crude ethyl chaulmoograte with 4% creosote from Calcutta	93.2	30	2/5	35-40	9/10	3-48 hr.	Pulmonary and gastro-enteric congestion	Markedly depressed with increased respiratory rate and fibrillary twitchings
Cottonseed oil with 4% creosote, control	—	30	2/5	35-40	7/10	1-24 hr.	Congested gastro-enteric tract	Markedly depressed with increased respiratory rate and fibrillary twitchings
Cottonseed oil, control	—	60	0/5	60	—	—	—	—

higher content of ethyl chaulmoograte; (2) iodizing ethyl chaulmoograte with 0.5% iodine has little effect on toxicity; (3) addition of 4% creosote increases the toxicity, commensurate with the amount of creosote present; (4) the degree of unsaturation of crude ethyl chaulmoograte is not a sufficiently sensitive index of toxicity to be reliable; and (5) if subcutaneously administered ethyl chaulmoograte exerts its toxic effect through liberation of Na chaulmoograte into the circulation, the summation of rates of hydrolysis and diffusion cannot much exceed that causing a disappearance of 2 millimols per Mol of the injected ester per hour, since Na chaulmoograte kills rats in intravenous acute doses[3] of 0.1-0.125 gm./kg.

Intensive treatment of leprous humans with ethyl chaulmoograte may give rise to a sterile tetanus in rare instances, perhaps related to the type of terminal convulsions seen in rats after administration of a single lethal dose. Conceivably, then, from this observation and the autopsy findings reported in Table I, death may occur from respiratory failure caused either by multiple emboli in the lungs or by prolonged central depression following a relatively short period of excitation, or from reduction of diffusible calcium in the plasma through formation of Ca dichaulmoograte, leading to tetany. No conclusions can be drawn here as to the importance of these and other factors in producing types of injury seen on chronic administration of chaulmoograte[4] nor as to the possible relative therapeutic rank of the different preparations, aside from an application of this acute toxicity data as a rough index of the absolute amount of ethyl chaulmoograte present.

7650 C

Attempts to Isolate Dihydroxy-pyrrol-Alanine from Gelatin Hydrolysates.

OLIVER H. EMERSON AND CARL L. A. SCHMIDT.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Van Slyke and Hiller[1] and Van Slyke and Robson[2] have reported the presence in gelatin of a new amino acid which is precipitable by phosphotungstic acid. On the basis of the elementary analysis of

[3] Emerson, G. A., Anderson, H. H., and Leake, C. D., *Arch. Internat. de Pharmacodyn. et de Therapie*, 1934, **48**, 247.

[4] Frazier, C. N., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 44.

the copper salt, a positive test for a pyrrol group, and the ratio of amino to total nitrogen, the product was considered as being probably dihydroxy-pyrrol-alanine. According to the criteria which have been suggested by Vickery and Schmidt,[3] the evidence brought out by Van Slyke and his coworkers is not sufficient to consider this substance as one of the accepted amino acids. The experiments herein described deal with a number of attempts to isolate and identify the product described by Van Slyke and his coworkers.

Three lots of about 1 kilo each of a good grade of gelatin were treated essentially as described by Van Slyke and his coworkers except that the bulk of the arginine was first removed by means of flavianic acid. On decomposing the phosphotungstic acid precipitate a product was obtained which was extremely soluble in water but no evidence was obtained to the effect that this substance was dihydroxy-pyrrol-alanine. In the first experiment there was present in this fraction 884 mg. of nitrogen of which 340 mg. were liberated by treatment with nitrous acid in 3 minutes and 374 mg. in 30 minutes. Flavianic acid gave no precipitate with a fairly concentrated solution even on standing for 24 hours at 0°. The substance represented by the amino nitrogen was almost quantitatively precipitated by rufianic acid and by picrolonic acid. The rufianate was amorphous and decomposed without melting. The picrolonate was crystalline and fractional recrystallization from water and dilute alcohol resolved it into at least 3 fractions which differed widely in melting point, solubility, and somewhat in crystal form. None of these fractions could be isolated in pure form.

In the second experiment it was likewise not found possible to isolate a pure product. The evidence pointed to the belief that the product was a mixture which contained a considerable amount of peptids since the amino nitrogen increased over 50% when the substance was hydrolyzed in a sealed tube with 6 N HCl at 120-140° for 4 hours.

In the third experiment a kilo of Coignet's gold label gelatin was hydrolyzed by boiling 24 hours with 8 normal sulfuric acid. The bulk of the sulfuric acid was removed by addition of $Ca(OH)_2$ and the remainder with $Ba(OH)_2$. The small amount of $Ca(OH)_2$ in solution was precipitated by means of oxalic acid. The bulk of the arginine was precipitated with flavianic acid. In order to eliminate

[1] Van Slyke, D. D., and Hiller, A., *Proc. Nat. Acad. Sci.*, 1925, **7**, 185.

[2] Van Slyke, D. D., and Robson, W., PROC. SOC. EXP. BIOL. AND MED., 1925, **23**, 23.

[3] Vickery, H. B., and Schmidt, C. L. A., *Chem. Rev.*, 1931, **3**, 169.

the possibility that the substance sought had been carried down by the crude arginine flavianate, the latter substance was recrystallized from 2 liters of 9% H_2SO_4 and the mother liquor was added to the main solution. The excess flavianic acid contained in the main solution was extracted with butyl alcohol. Since the latter solvent had extracted a considerable amount of amino nitrogen which could not be removed by washing with 10% H_2SO_4, it was evaporated to dryness *in vacuo* and the residue was taken up in a small amount of hot water. The flavianic acid was precipitated by the addition of HCl, the solution was diluted with 5% H_2SO_4, and the bases which had been extracted along with the flavianic acid were precipitated with phosphotungstic acid. The precipitate was washed and added to the bulk of the precipitate which was obtained by the addition of phosphotungstic acid to the main solution. The precipitated phosphotungstates were decomposed with $Ba(OH)_2$, histidine and the small amounts of arginine remaining in the solution were precipitated with silver sulfate and $Ba(OH)_2$ and the lysine was removed as the picrate in accordance with the procedure described by Vickery and Leavenworth[4] and Vickery and Block.[5] The alcoholic filtrate from the lysine picrate was evaporated to dryness and the residue was dissolved in water to which about 15 cc. of H_2SO_4 were added. The picric acid was extracted with toluene. The diluted solution was again treated with phosphotungstic acid.

There was present in the solution from the decomposition of this second phosphotungstate precipitate 1.655 gm. of nitrogen of which 480 mg. represented amino nitrogen. The solid material in this solution was fractionated with absolute alcohol. The alcohol soluble portion contained 1.360 gm. of nitrogen of which 391 mg. were in the form of amino nitrogen, while the alcohol insoluble fraction contained 139 mg. of nitrogen of which 40 mg. represented amino nitrogen. Further fractionation and identification of these two products are shown in Diagram I. A few comments on the diagram deserve consideration.

The precipitation of the reineckate, the decomposition of the precipitate, and removal of the inorganic ions were carried out in accordance with the procedure of Kapfhamer and Eck.[5] The reinecke precipitate from the alcohol soluble fraction contained a considerable amount of arginine whose removal by the silver method was not wholly successful. This necessitated treatment with flavianic

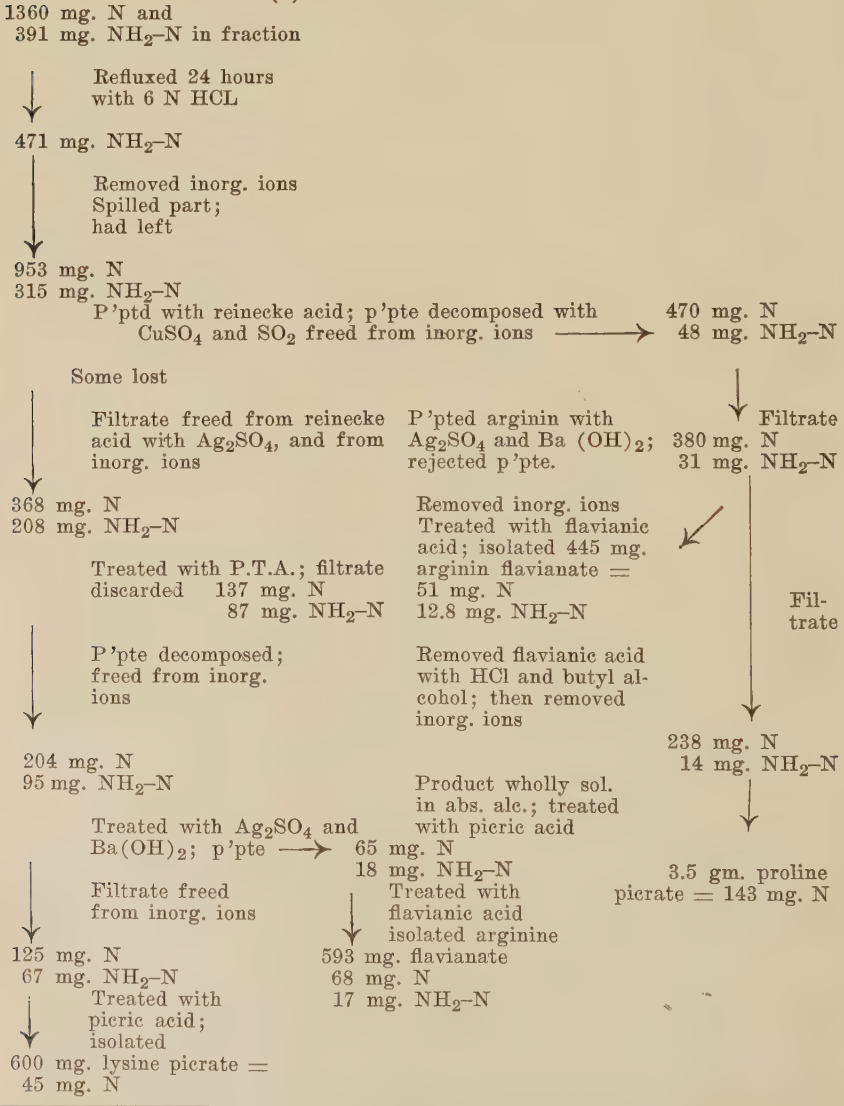
[4] Vickery, H. B., and Leavenworth, C. S., *J. Biol. Chem.*, 1929, **83**, 532. Vickery, H. B., and Block, R. J., *J. Biol. Chem.*, 1931, **93**, 105.

[5] Kapfhamer, J., and Eck, R., *Z. physiol. Chem.*, 1927, **170**, 294.

acid. To test the purity of the arginine flavianate it was recrystallized from dilute H_2SO_4 and then boiled with water to decompose any arginine diflavianate. The nitrogen values of the purified product corresponded very closely to that calculated for arginine flavianate. The proline picrate contained practically no amino nitrogen. It melted at 149°. Towne[6] reports 148°, while Kapfhamer

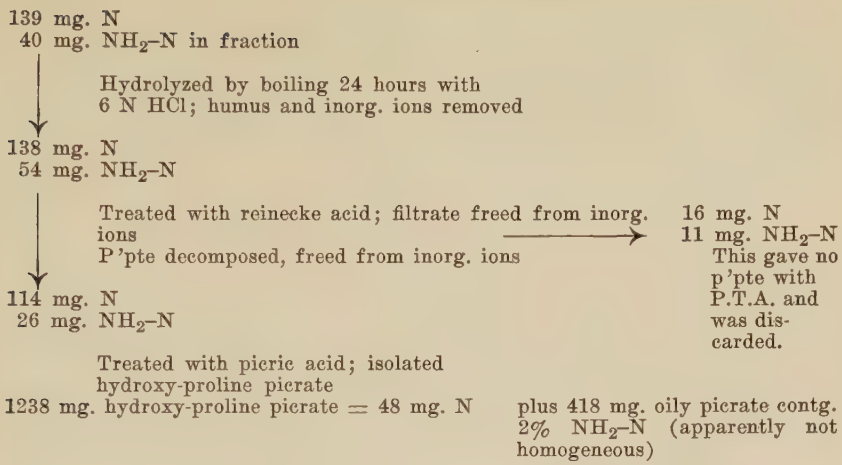
DIAGRAM I.

(a) Alcohol Soluble Fraction.



[6] Towne, B. W., *Biochem. J.*, 1928, **22**, 1083.

(b) Alcohol Insoluble Fraction



and Eck found 154°. The nitrogen content was 16.18%. The first crop of lysine picrate contained 18.47% of nitrogen and exploded at 257°. The hydroxyproline picrate, after recrystallization from water and then from an alcohol ether mixture, melted at 180°. Kapfhamer and Eck give 188°. The nitrogen content was 15.6%. It was practically free from amino nitrogen.

It is evident from the data presented that no substance corresponding to the empirical composition of dihydroxy-pyrrol-alanine was isolated in the 3 experiments. The fraction in which this compound should have been present contained some peptids. After hydrolysis, in addition to arginine and lysine, considerable amounts of proline and hydroxyproline were isolated. This is not surprising since both of these amino acids are in part precipitated by phosphotungstic acid. Since both the reineckate and the picrate of these 2 amino acids are relatively soluble, the amounts present in the hydrolysate were doubtless considerably greater than were isolated. The discarded filtrate which contained 137 mg. nitrogen of which 87 mg. were in the form of amino nitrogen probably contained either peptids or unprecipitated hexone bases in addition to some monoamino acids. If dihydroxy-pyrrol-alanine was present in any of the fractions of the gelatin hydrolysate, the amount must have been relatively small.

It should be mentioned that the empirical formula of dihydroxy-pyrrol-alanine differs from that of glycyl-hydroxyproline only by having two more hydrogen atoms. On hydrolysis there should be no increase in amino nitrogen. It is not impossible that this peptid may have been mistaken for an apparently new amino acid and

especially since the pyrrol test is not a specific test for this group. Gelatin is known to be difficultly hydrolysable. While the present experiments do not wholly deny the existence of the amino acid described by Van Slyke and his associates, they nevertheless indicate that more decisive proof must be advanced before the substance can be considered as an accepted amino acid.

7651 C

Necessary Concurrence of Thyroid in the Marked Adrenal Cortical Hypertrophy Following Beef Anterior Pituitary Implants.

MORVYTH MC QUEEN-WILLIAMS. (Introduced by Herbert McLean Evans).

From the Anatomical Laboratory, University of California.

Emery and Atwell[1] showed that the administration to normal animals of extracts of whole sheep pituitaries provoked a marked increase in adrenal weight due predominantly if not exclusively to the hypertrophy of the cortex. Independently, we had obtained the same effect in rats from implants of beef anterior pituitary tissue. The present communication reports a striking diminution in this effect when a preceding ablation of the thyroids has been performed.

Fresh beef hypophyses were washed in alcohol before dissection, and to eliminate all chance of infection, both merthiolate (1:10,000) and hexylresorcinol (1:13,000) were added to many of the batches immediately after grinding. Of 214 adult male and female rats, some of which were unilaterally adrenalectomized, 41 had been previously hypophysectomized, 69 including many first thyroidectomized received pituitary implants, 67 including thyroidectomized were left untreated, and a group of 37 normals was sacrificed and right and left adrenals weighed separately.

Results. 1. Implants of adequate amounts of finely ground beef hypophyses, whether freshly prepared or stored at 0°C. for several months, induced marked adrenal hypertrophy in every case in which the animal possessed intact thyroids, but had little or no effect on thyroidectomized rats (Tables I and II).* 2. Two grams of an-

[1] Emery, F. E., and Atwell, W. J., *Anat. Rec.*, 1933, **58**, 17.

* In 37 *normal* male rats (64 to 82 days) the *left* adrenals averaged 18.3 mg., the right 16.0 mg.; only once did the right exceed the left. Consequently, in all the experiments only *left* unilateral adrenalectomies were performed.

Autopsies of unilaterally adrenalectomized rats revealed that the *right* adrenal always exceeded the left, extirpated 5 days to 4 months previously. More extended study on this compensatory hypertrophy is contemplated.

TABLE I.

Showing That Adrenal Weight Increase Following Beef Hypophyseal Implants in Rats Is Greatly Diminished in Absence of Thyroid.

Exp.	No. Rats (Male, except Group 1)	Approx. Age at Autopsy (Days)	Total days since Thyroid-ectomy	Total Mg. Beef Ant. Pituitary Implanted	No. Implants	Aver. Wt. of 2 Adrenals (Mg.)				Aver. Wt. of 2 Thyroids (Mg.)	
						Untreated	Implanted	Thyroid-ectomized Untreated	Thyroid-ectomized Implanted	Untreated	Implanted
1	2	190	—	2000	5	20*	53*	—	—	—	—
2	20	65	40	2000	4	34	73	31	35	33	25
3	4	60	35	1850	5	36	62	28	34	30	27
4	4	45	—	1300	1	19	29	—	—	21	16

*Weight of one adrenal.

TABLE II.

Showing That Adrenal Weight Increase Following Beef Hypophyseal Implants Is Also Diminished in Unilaterally Adrenalectomized Rats in Absence of Thyroid.

Exp.	No. Rats (Male)	Approx. Age at Autopsy	Total Days Elapsed after Left Unilat. Adrenalectomy	Total Days Elapsed after Thyroid-ectomy	Total Mg. Beef Ant. Pituitary Implanted	Av. % Increase in Adrenal Wt.			Aver. Wt. of 2 Thyroids (Mg.)		
						Untreated	Implanted	Thyroid-ectomized Implanted	Normal	Untreated	Implanted
1	11	154	12	125	2050	42	170	56	—	47	31
2	3	140	25	108	1975	40	100	85*	—	43	34
3	10	80	30	50	1850†	97	281	105	31	32	26
4a	10	80	17	60	2600	38	137	57	29	27	21
4b	3	80	17	60	1450	—	73	20	—	28	21
5a	3	63	13	33	1900	—	91	43	24	—	20
5b	2	63	13	33	2000	—	105	54	—	—	21
5c	1	63	5	—	1500	—	74	—	—	—	16
5d	3	63	5	—	550	14	53	—	—	24	20
5e	3	63	5	—	100-250	—	44	—	—	—	20
6	3	60	10	30	570	24	56	27	—	22	—
7	6	45	5	—	—	20	—	—	—	22	—

*One mg. fragment of thyroid remaining.
†Administered 5 implants; in the other groups 2 implants were made.

terior pituitary implants increased the adrenal weight of rats with intact thyroids to at least double that of the controls, larger or smaller amounts producing proportionate effects. 3. In one case where 60 days had elapsed between the last treatment and autopsy, the adrenal had maintained its increased size, being palpable during the whole period, and weighing at autopsy 80 mg., that of a sibling control being 26.5 mg. 4. The adrenal cortex was chiefly affected by the preparations employed. The hypertrophied adrenals, stained with Mallory-Azan, exhibited the zona reticularis and fasciculata cells greatly increased in size and, especially in the former zone, highly vacuolated, indicating lipoid increase.

The extremely slight response of *thyroidectomized* animals to beef pituitary implants is excellent evidence that the adrenal hypertrophy in rats with intact thyroids is attributable to the action of a pituitary hormone acting by way of the thyroid and is not due to bacterial or to foreign protein influence. Possibly the anterior hypophysis possesses 2 hormones, one acting directly on the adrenal and the other acting on the adrenal via the thyroid. Evidence for the direct hypophyseal action is offered by (1) the maintenance of adrenal weight in thyroidectomized rats; heavy adrenals (av. 73.5 mg.) were found in 14 female rats thyroidectomized for 12 to 20 months; all had large hypophyses (av. 17.0 mg.). (2) Compensatory adrenal hypertrophy occurred in unilaterally adrenalectomized thyroidectomized animals, but not in hypophysectomized rats that had been unilaterally adrenalectomized 8 to 21 days before autopsy; in 41 rats (av. 274 gm.) hypophysectomized by the writer, each adrenal degenerated to 4.6 mg. (av.). It would appear that the pituitary hormone which acts through the thyroid exists in considerable amount in the normal beef hypophyses implanted, but that the directly acting hormone is not present to any great extent.

7652 C

Antibody Response to H. Pertussis Undenatured Bacterial Antigen.*

JOHN J. MILLER, JR.,† ALCOR S. BROWNE AND NERISSA MC CREA.
(Introduced by A. P. Krueger.)

From the Department of Bacteriology, University of California, Berkeley.

The rise in complement fixing antibodies in the sera of rabbits following a single injection of *H. pertussis* undenatured bacterial antigen (Krueger *et al.*)[1] was determined. The method used was that described (Miller[2]) in connection with a study of other types of *H. pertussis* extracts. Comparable amounts on the basis of total nitrogen content were used—approximately 0.05 mg./cc. In 6 rabbits tested the appearance of complement fixing antibodies was demonstrated on the 6th to the 20th day after injection. The rise in their titre thereafter was gradual. This response to *H. pertussis* U. B. A. differs from that of *H. pertussis* extracts previously described,[2] in that it is slower and more variable.

The antibody response to repeated injections of *H. pertussis* U. B. A. was then observed in 10 children. None had a history of whooping cough or *H. pertussis* vaccination. They were members of a group of 50 subjected to 4 immunizing injections of *H. pertussis* U. B. A. At weekly intervals there were given subcutaneously 0.5 cc., 1.0 cc., 1.0 cc., and 2.0 cc. of a preparation containing 8 mg. of nitrogen/100 cc.

Complement fixing antibodies were demonstrated in the sera of 4 children on the seventh day after completion of the injections and a fifth sera was found positive on the eighteenth day. On the sixty-fourth day 3 of these remained positive. Agglutinins were demonstrated in 5 sera on the seventh day, in 9 sera on the eighteenth day and in only 3 sera on the sixty-fourth day. Precipitins were demonstrated in 3 instances on the eighteenth day and in 9 instances on the sixty-fourth day.

Conclusion. Herein it is demonstrated that *H. pertussis* U. B. A., an undenatured extract prepared by mechanical disruption of Phase I *H. pertussis* bacilli and subsequent filtration through a collodion membrane, is able to stimulate the formation of complement fixing antibodies, agglutinins and precipitins.

* Supported by Grant-in-Aid of Research from Eli Lilly and Company.

† National Research Council Fellow in Medicine.

1 Krueger, A. P., Nichols, V. C., and Frawley, J. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1097.

2 Miller, J. J., Jr., *J. Immunol.*, 1934, **26**, 247.

New York Meeting.

New York Academy of Medicine, November 21, 1934.

7653 P

Active Immunization of Children Against Poliomyelitis with Formalin Inactivated Virus Suspension.*

MAURICE BRODIE.

From the Bureau of Laboratories, Department of Health, New York City, and Department of Bacteriology, New York University and Bellevue Medical School.

In previous communications[1] it was pointed out that definite immunity could be developed against the virus of poliomyelitis using virus rendered non-infective by formalin. However, the amount of formalin used produced considerable skin irritation. Since then it has been shown that virus suspensions inactivated with 0.1% formalin, at incubator temperature, were also antigenic for monkeys, and at the same time, gave practically no skin irritation. The majority of the animals showed both humoral immunity, as tested by the neutralizing power of the serum for the virus of poliomyelitis; and tissue immunity, that is resistance to intracerebral inoculation of active virus.

The inactivated antigen produced no reaction whatsoever, neither symptoms, temperature rise nor cerebrospinal fluid changes developed upon repeated inoculations of large doses given both intracerebrally and intraperitoneally, each dose representing the equivalent of thousands of infective doses of living virus. Moreover, during vaccination the animal suffered no untoward local or systemic reactions, so it was felt that the vaccine could be given to humans with perfect safety.

However, before giving it to children, it was deemed advisable to try it upon ourselves, not that we had any misgivings about the possibilities of infection, but rather to determine whether the vac-

* This work was aided by grants from the New York Foundation and the Rockefeller Foundation.

[1] Brodie, M., *Science*, 1934, **79**, 594; *J. Immunol.*, in press

cine produced any disagreeable local or general reactions. Accordingly, 6 volunteers from the Bureau of Laboratories, Department of Health, New York were given 5 cc. doses of a 10% virus suspension, inactivated with 0.1% formalin for 16 to 48 hours. Three were given one dose, two, 2 doses and the third, 3 doses. The second inoculation was given 11 days after the first, and the third, 8 days after the second. After inoculation there was some soreness at the site of injection, lasting but a few minutes and probably due to the formalin. Three of those injected had some induration, which lasted but a few days and was not painful or uncomfortable. In no instance was there any systemic reaction.

The blood serums of all 6 obtained before immunization were tested for antibody and the amount determined by careful titration. A preliminary test was carried out upon the serums of the 3 who had more than one dose of vaccine and some increase in antibody was demonstrable.

As it was now evident that the vaccine could be administered with perfect safety it was given to a series of 12 children, whose ages ranged between 1 and 6 years. The virus suspension used for preparation of the vaccine was cultured aerobically and anaerobically before it was treated with 0.1% formalin for 16 hours, 12 hours being the time required to inactivate the virus. Five received a single dose of 5 cc., the others were given a second dose, either 11 or 13 days later. One to 2½ cc. was given intracutaneously, the remainder subcutaneously. The material was injected into the skin of the abdominal wall.

The children were carefully observed for local and general reactions and temperatures were recorded 4 times daily. There was no apparent general reaction or discomfort and at no time any febrile manifestations that could be attributed to the vaccine. The local reaction was negligible, consisting only of some induration in those receiving the larger amounts intracutaneously. The first dose did not render the children sensitive to the second dose.

In order to determine the degree of immunity produced by the vaccine, in each instance the antibody or antiviral content of the serum was determined quantitatively, by estimating the number of minimal completely paralyzing (M.C.P.) doses of virus the serum neutralized. The M.C.P. dose representing the smallest amount of virus-containing tissue that will produce a complete and rapid paralysis in a monkey of 2.5 to 4 kg. within 13 days.

All the children showed, prior to immunization, a small amount of neutralizing substance, too little to be of any significance. After

the vaccine each showed an appreciable increase. As far as the tests have been completed the blood serums of 6 children neutralized between 100 and 200 and those of the other 6 between 200 and 500 additional infective doses of virus.

Of course we have no index as to the level of immunity required to protect the children against the natural infection, nor whether the immunity obtained in the present series is sufficient. However, inasmuch as the humans responded better to the antigen than did the experimental animals and inasmuch as the monkeys injected with formalized virus showed a relatively high tissue immunity, sufficient to withstand in most cases intracerebral inoculation of virus, it is quite likely that the humans have an appreciable tissue immunity. By analogy with louping-ill,[2] a disease of sheep that is quite analogous to poliomyelitis, it should be ample to protect against natural exposure to the virus, for formalized louping-ill virus was unable to protect against intracerebral inoculation, yet it did so against the natural disease. However, the ultimate proof of the protective value of the vaccine must be established in a properly controlled series vaccinated in an epidemic area.

7654 P

Vascular Action of Fresh Urine and Extracts Thereof.

IRVINE H. PAGE.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

Human urine and extracts of urine have been known for some time to contain both pressor and depressor substances.[1] Attempts have been made to associate them with the presence of arterial hypertension with small success. Since the properties of these substances are little known, we have undertaken a study of them.

Cats anesthetized with ethyl urethane (7-10 cc. of a 25% solution given subcutaneously) or ether were employed as test animals. Blood pressure was recorded from the femoral artery and the vagus nerves were severed. Injections of the warmed fluid were made

[2] Gordon, W. S., *Proc. Roy. Soc. Med.*, 1934, **27**, 11; *Veterinary Rec.*, 1934, **14**, 1.

[1] Abelous, J. F., and Bardier, E., *J. d. Physiol. et path. gén.*, 1908, **10**, 627; 1909, **11**, 34.

into a femoral vein. Forty-one animals have been employed for the experiments.

Injections of 5 cc. of fresh normal urine usually produced elevation of blood pressure of from 6 to 20 mm. of Hg. Boiling the urine for 1-3 minutes did not affect the pressor action, nor did preliminary atropinization of the animal. Mixtures of urea and sodium chloride, or sodium acid phosphate of the same specific gravity as the urine, did not appear to duplicate the vascular action of urine.

Often the same test animal which yielded a pressor response to normal urine exhibited depression of the blood pressure on injecting the same urine at another time during the course of the experiment. Some animals exhibited only depressor responses. We have been unable to predict which response will occur, though the pressor was the more common. At times the animals became entirely refractory to urine injections. The variability of response led us to suspect that the functional intactness of the central nervous system was essential for a vascular response to be elicited. Decerebrate and pithed cats were found to be refractory to urine injections, whereas the same animals before pithing had responded actively. It appeared, then, that the vascular action of urine was not peripheral, but central.

An attempt has been made to separate the pressor and depressor substances from urine by extraction methods. It was found that ethyl acetate extracts much of the pressor substance from urine. One liter lots of urine were extracted with about 200 cc. of freshly distilled solvent and the solvent removed under vacuum. The residue was extracted with (a) ether (50 cc.) followed by, (b) alcohol (50 cc.) and the remaining insoluble material dissolved, (c) in water (10 cc.). The ether and alcohol were removed under vacuum and the residues dissolved in water (10 cc.). Such extracts (a and b) were usually found to depress the blood pressure from 12 to 40 mm. Hg., while the water solution of the residue (c) elevated the pressure 20 mm. or more. Thirty-two urine specimens have been examined in this manner.

Acetone extracts of urine have been prepared by saturating urine with salt and layering with acetone. After removal of the acetone the residue was separated into ether, alcohol and water soluble fractions. Essentially the same vascular responses were observed as when ethyl acetate was used as solvent. Adjusting the pH of the urine to acid and alkaline reactions did not appear to aid in the extraction.

Capps, Ferris, Taylor and Weiss (personal communication) have studied extracts of urine prepared by adsorption on Norit and elu-

tion with acetone and alcohol. They found that acetone removed from the Norit more pressor substance than did alcohol extraction. The pressor substance apparently acted centrally because its action paralleled that of alpha lobelin, a central acting drug. Contrary to Bohn and Hahn[2] they found no relationship between the amount of pressor substance in the urine and the presence or absence of hypertension in the patient.

7655 C

Potentials in Embryo Rat Heart Muscle Cultures.

B. M. HOGG, C. M. GOSS AND K. S. COLE. (Introduced by H. B. Williams.)

From the Departments of Physiology and Anatomy, Columbia University.

As far as we have been able to discover, there have been no published investigations of action potentials in heart muscle cultures. This is a report on a method which it is hoped will furnish information on the mechanism of irritable tissues in general and particularly those which are excited spontaneously.

In general, the potential difference between the micro-electrodes making contact with a muscle culture is impressed on the grid of a vacuum tube and measured by the deflection of a string galvanometer in the plate circuit.

The cultures were prepared by the usual hanging drop technique. Small pieces of the ventricle of 16-day rat embryos were explanted in a medium of rat blood plasma and rat embryo extract. The ages of the cultures varied from 2 days to 2 months. The present records were taken from the older cultures containing outgrowths of muscle which had differentiated in the manner described by Goss[1] and were presumably without nervous tissue elements. Spontaneous contraction occurs in practically all cultures at one time or another and may be rhythmic or irregular. There is a great variation in the duration of the resting and acting periods and also in the frequency of rhythmic activity.

The electrode systems are micro-pipettes, 2-5 mu in diameter at the tip, with chloride coated silver wire coils placed in the large ends and the whole filled with mammalian Ringer solution. These pipette

[2] Bohn, H., and Hahn, F., *Z. f. klin. Med.*, 1933, **123**, 558.

[1] Goss, C. M., *Arch. f. Exp. Zellforsch.*, 1932, **12**, 233.

electrodes are mounted in micro-manipulators[2] and enter the moist chamber, on which the culture cover slip is placed, through a water trap as used by Chambers.[3] Such an electrode system has a high electrical resistance which precludes the direct use of a rapid recording instrument even if polarization difficulties could be overcome. The electrodes are connected to the grid circuit of a type 32 vacuum tube, operating at its free grid potential so as to draw a minimum of grid current. The Einthoven string galvanometer as used has a response time of about 0.01 sec. and is connected in the plate circuit of the tube. The steady plate current is by-passed through the usual Dowling shunt. A standard tuning fork driven time wheel giving time in 0.04 sec. is used and the camera is of the usual electrocardiograph type. To eliminate interference all electric power lines in the room are disconnected, the camera driven by an air motor, and a one microfarad condenser connected across the tuning fork contacts.

The ground electrode was usually placed in the culture medium at a short distance from the culture and the grid electrode was used for exploration.

As the exploring electrode approached the culture, a small but increasing negative potential relative to ground was found. Upon traversing the culture, one and sometimes 2 or 3, well demarcated areas about 10-20 mu in diameter were found where the negative potential was markedly higher, sometimes as much as 20-30 millivolts. These areas seemed sometimes to lie in the vicinity of elongated cells.

With the exploring electrode near one of these negative centers, contraction of the culture is coincident with a positive "action" potential, *i. e.*, a decrease of the negative resting potential. The record of Fig. 1a is from a particularly sensitive culture which was stimulated to activity by tapping the microscope with the electrode in position near an active center. In another culture, Fig. 1b, stimulation was effected by moving the electrode slowly toward an active center, showing an increase of the negative resting potential before the initiation of activity. After stimulation, the action potentials assume a characteristic form which is repeated at a constant or slightly decreasing frequency until quiescense. The form is that of a quick positive rise followed by a less rapid and rather uniform fall until near the resting potential which it reaches more slowly as

[2] Microdissection and microinjection—Microscopical Technique, 1929. R. Chambers, edited by McClung, Paul Hoeber, N. Y., pp. 39-73.

[3] Cohen, B., Chambers, R., and Reznikoff, P., *J. Gen. Phys.*, 1928, **11**, 585.

a negative maximum. There is then a slight positively directed rise before the initiation of the next beat. When activity ceases, the potential of the final beat apparently follows the same course except that it returns to the resting potential after this small positive rise (or decrease in negativity) instead of going into the next contraction. This gives rise to a characteristic final or "after-potential" as shown in Figs. 1a and 1b.

On one occasion in a slowly beating culture—60 per min.—somewhat similar after-potentials were noted following each response

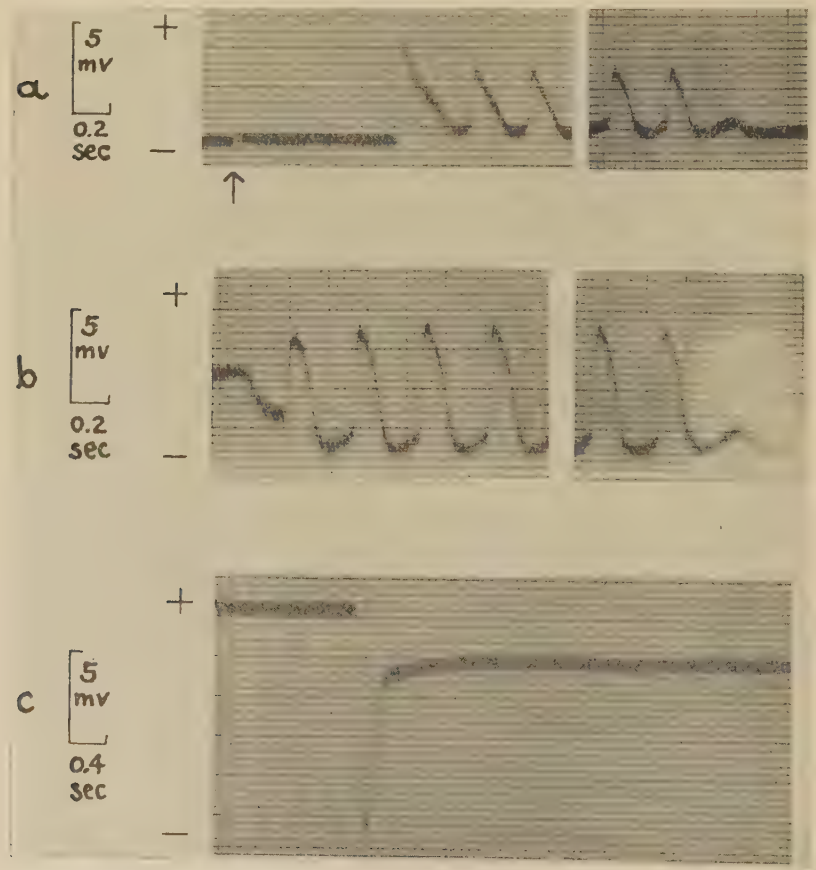


FIG. 1a. Potential difference between exploring electrode near negative center and ground electrode in culture medium when rhythmic contraction was initiated by tapping the microscope and ceased spontaneously.

FIG. 1b. Same as Fig. 1a, except that activity was initiated by moving exploring electrode nearer negative center.

FIG. 1c. P. D. between exploring electrode and ground electrode in medium when the former was forced through the surface of a single cell of a resting culture. A rapid darkening of the cell indicated its death.

which seemed to be coincident with mechanical relaxation or with the reflected wave seen in the slow-motion pictures of contracting cultures.[4] Close examination of the records would seem to suggest that such after-potentials occur with each beat in the more quickly beating cultures (150 per min.) but are incorporated in the record of the following beat. Faster galvanometer and film speeds would be necessary to demonstrate this.

Efforts to obtain diphasic responses were unsuccessful when the electrodes were placed one to 2 cell lengths apart along a 2 to 3 cell strand of contracting muscle. The only diphasic record was obtained by putting one electrode near each of 2 negative centers which were slightly out of phase.

With the unaided eye it is impossible to say whether the electrical centers serve also as centers for the spread of mechanical contraction or not. No apparent difference was found in the action potentials of non-striated cultures and those in which striation was almost complete.

An attempt was made to register the potential changes across the membrane of a single contracting cell, but failed because the excessive vibration of the inserted electrode caused almost immediate death of the cell as judged by refractive properties. The rather characteristic "death wave", Fig. 1c, shows a sudden large negative rise (which may be 20 millivolts) followed by an almost equally rapid fall to about half this value or less. Small variations on some of these records were attributed to contractions of neighboring cells.

7656 P

Note on the Metabolism of Copper in Splenectomized Rabbits.

MARTA SANDBERG AND OLIVE M. HOLLY.

From the Laboratory Division, Montefiore Hospital, New York City.

In previous studies[1] it was found that removal of the spleen in albino rats free from *Bartonella muris* infection is followed by an increased elimination of copper, which produces a negative copper balance. In a study of the metabolism of rabbits before and after splenectomy we found that removal of the spleen causes an increase in copper excretion in this species as well.

[4] Goss, C. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 292.

[5] Goss, C. M., *Arch. f. Exp. Zellforsch.*, 1933, **14**, 175.

[1] Sandberg, M., and Perla, D., *J. Exp. Med.*, 1934, **60**, 395.

Two rabbits were splenectomized at the age of 2½ months, after a foreperiod of 3 weeks, while another one was kept as a control under identical conditions. They were given alfalfa hay, oats and copper-free water, which was also used for all chemical determinations and for washing everything that came in contact with the animals. Urine was collected daily, feces twice a week. The urine was analyzed for total nitrogen (Kjeldahl), total sulphur (Benedict), total and inorganic sulphates (Folin), calcium (volumetric), phosphorus (Fiske-Subbarow), and copper.[2] Feces, hay and oats were analyzed for total nitrogen, calcium, phosphorus, copper and iron.[3]

Our experiments show that splenectomy causes no change in the metabolism of nitrogen, sulphur, phosphorus and iron in rabbits. The calcium excretion proceeds unchanged after splenectomy, which also tends to show that the spleen is not involved in the regulation of calcium metabolism.

While the excretion of copper in the intact animal was remarkably constant, it increased in the splenectomized animals a week after the operation. The increase takes place in the feces, since the greater part of copper is excreted by the gut. The excretion of copper by the kidney does not seem to be influenced by splenectomy, though a larger amount of the total copper excreted is found in the urine in rabbits than in rats. As shown in the table, the copper intake on a diet of hay and oats is insufficient to maintain a positive copper balance in a growing rabbit, but the loss of copper from the body

TABLE I.
Daily Average of Copper Excretion per Week.

	Splenectomized Rabbit					Normal Rabbit				
	Urine	Feces	Total Excretion	Intake	Retention	Urine	Feces	Total Excretion	Intake	Retention
1934	mg.	.mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
May 7-13	.156	.437	.593	.536	—.057	.202	.456	.658	.538	—.120
14-20	.156	.446	.602	.548	—.054	.218	.443	.661	.543	—.118
21-27	.164	.438	.602	.543	—.059	.223	.450	.673	.544	—.129
28-*										
June 3	.168	.453	.621	.557	—.064	.232	.455	.687	.557	—.130
4-10	.164	.545	.709	.559	—.150	.226	.451	.677	.558	—.119
11-17	.113	.868	.982	.544	—.438	.230	.452	.682	.549	—.133
18-24	.114	.653	.767	.540	—.227	.220	.453	.673	.546	—.127
25-										
July 1	.155	.794	.949	.545	—.404	.214	.448	.662	.546	—.117
2-8	.144	.573	.717	.538	—.179	.224	.446	.670	.549	—.121
9-15	.146	.565	.711	.542	—.169	.232	.450	.683	.553	—.130

*Splenectomized May 28th.

[2] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

[3] Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1926, **67**, 43.

increases considerably after splenectomy. This further supports the evidence presented in our study of the copper metabolism of rats regarding the rôle of the spleen in the utilization of copper.

7657 P

Shwartzman Phenomenon with B. Pertussis Culture Filtrates.

LEWIS HENRY KOPLIK. (Introduced by Gregory Shwartzman.)

From the Laboratories of the Mount Sinai Hospital, New York.

Attempts to reproduce the Shwartzman phenomenon with *B. pertussis* have been reported by several authors. (Gross,[1] Shwartzman,[2] Mishulow, Mowry and Scott.[3]) However, the potency of the filtrates seemed to have varied for unknown reasons and always remained low as compared to similar preparations from *B. typhosus*,[4] meningococcus[5] and other organisms.[6]

During some work with serum neutralizations of *B. pertussis* toxic substances it occurred to the author of this paper to employ Toomey and McClelland's brain veal infusion medium[7] for the preparation of the toxic factors necessary for the production of the Shwartzman phenomenon.

In these experiments the brain medium was prepared essentially according to the method of Toomey and McClelland with various peptones (neopeptone, proteose-peptone (Difco), Witte's peptone). The H-ion concentration was adjusted either to pH 7.3 or 7.8. The media were seeded with 24-hour-old cultures on "chocolate agar" slants. The strain used was subcultured for at least 3 successive days preceding these inoculations.

The organisms grew luxuriantly in the brain media similar to Toomey and McClelland's description. The strain employed was M 12 (group B), kindly supplied to us by Miss Mishulow of the New York City Board of Health Laboratories.

[1] Gross, Louis, personal communication.

[2] Shwartzman, Gregory, *J. Exp. Med.*, 1930, **51**, 581.

[3] Mishulow, Lucy, Mowry, Isabelle W., and Scott, Eleanor B., *J. Immunol.*, 1930, **19**, 227.

[4] Shwartzman, Gregory, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 843.

[5] Shwartzman, Gregory, *J. Inf. Dis.*, 1929, **45**, 232.

[6] Shwartzman, Gregory, PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 207.

[7] Toomey, John A., McClelland, Joseph E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 34.

Berkefeld filtrates of 4, 7 and 17 days' brain medium cultures were tested in rabbits for the elicitation of the Shwartzman phenomenon. 0.25 cc. undiluted filtrate was injected intradermally into rabbits and followed in 24 hours by a single intravenous injection of the same filtrate undiluted or in varying dilutions. Comparative studies of the brain culture filtrate with "chocolate agar" culture washings were made. The results may be described in brief as follows:

It was possible to elicit the phenomenon under discussion in prepared rabbits by an intravenous injection of the brain filtrate in dilutions ranging from 1:40 to 1:400 and recently 1:1500. The chocolate media culture filtrates in our hands could never be diluted higher than 1:5, and usually were active only undiluted. It is interesting to note that some of the brain media culture filtrates when retitrated 2-4 months after preparation showed a rise in effective titer of 5 to 20 fold. This was not observed with "chocolate agar" culture filtrates. The optimum incubation period for brain infusion cultures seemed to be about 17 days, while 4 and 7 day cultures yielded filtrates of lower potency. The H-ion concentration in the range employed had no effect. Neopeptone and proteose-peptone (Difco) appeared superior to Witte's peptone.

This report seems to be justified because of the ease with which *B. pertussis* filtrates in large quantities can be obtained for the elicitation of the Shwartzman phenomenon; and because of the considerable potency of these preparations, *i. e.*, 5-20 times higher than with preparations hitherto described.

Experiments on specific serum neutralizations are under way in order to determine the nature and antigenicity of the toxic substances described above.

7658 P

Blood Changes Following Gastrectomy in Monkeys.

S. MILTON GOLDHAMER. (Introduced by C. C. Sturgis.)

From the Thomas Henry Simpson Memorial Institute for Medical Research, Ann Arbor, Michigan.

There has been considerable speculation as to whether the "pernicious anemia-like" blood picture following gastrectomy in man[1] is the result of the operation, or is an independent phenomenon. In-

vestigators, working with dogs,[2] rats,[3] and hogs,[4] have attempted to produce a macrocytic anemia by removal of the stomach, but in all instances a "secondary anemia" developed. This paper reports the observations on hematopoiesis following total gastrectomy in the *Macacus rhesus* monkey.

Eight monkeys (2 females and 6 males) averaging 8 pounds in weight, were used. Exact data concerning their ages were not available. The animals were observed at least 2 weeks before operation and during this period normal blood findings were recorded.[5] The first operation was performed on January 16, 1933, at which time the entire stomach was removed. The proximal end of the duodenum was sealed and the esophagus anastamosed with the jejunum. Similar operations were performed on 7 other monkeys.*

Six of the animals died within a period of 2 weeks after operation; 5 from aspiration pneumonia and atalectasis, and one from peritonitis. Of the 2 remaining monkeys, one (No. 2) lived for 10 weeks and the other (No. 4) lived for 23 weeks. Autopsies were performed on all animals immediately following death. In the animal living 10 weeks there was terminal circulatory failure with pulmonary congestion and edema, and necrosis at the cardiac end of stomach. In the animal living 23 weeks, a marked atrophic catarrhal enteritis was noted. One portion of the intestine showed extensive necrosis, diffuse phlegmon and early gangrene. There were small areas of diphtheritic colitis.

The monkeys were housed in the same environment before and after the operation. The diet was the same throughout, consisting primarily of green vegetables, fresh fruits, bread and sugarized milk. No anti-anemia therapy was instituted as it was desired to note the effect of gastrectomy on the functional activity of the hematopoietic system.

Three hours after the operation on Monkey No. 2 the red blood cell and white blood cell counts were markedly increased. This probably was due to dehydration and to contraction of the spleen. Within 72 hours a "secondary anemia" occurred, characterized

[1] Goldhamer, *Surg., Gynec. and Obst.*, 1933, **57**, 257.

[2] Ivy, Morgan and Farrell, *Surg., Gynec. and Obst.*, 1931, **53**, 2.

[3] Jung, Maison and Highstone, *Proc. Inst. Med.*, Chicago, 1933, **9**, 389.

[4] Maison and Ivy, PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 554.

[5] Hall, *Folia Haemat.*, 1929, **38**, 30. Scarborough, *Yale J. Biol. and Med.*, 1931, **4**, 199.

* All the operations were performed by Dr. Walter G. Maddock of the Department of Surgery, University of Michigan. The author wishes to express his gratitude to him for his assistance.

by a lower color index than normal, a moderate reduction in the red blood cells and a more pronounced reduction of the hemoglobin. During the 10-week period of life following the operation, the red blood cells fluctuated somewhat in number, reaching the lowest level on the day of death. The hemoglobin reached its lowest level 72 hours after the stomach was removed; it gradually increased for about 4 weeks, and then remained stationary. At no time did the hemoglobin return to a normal level. Red blood cell measurements at the time of death showed only a slight increase in the degree of dispersion and the average size of the red blood cells remained the same. The average uncorrected color index before the operation was 0.66. For about 8 weeks after the operation, the uncorrected color index was less, but gradually increased to this level. On the day of death the uncorrected color index and the increased dispersion in the red blood cell size were slightly suggestive that a macrocytic anemia might have developed if the animal had survived for a longer period of time but the evidence is very meager.

In animal No. 4, a "secondary anemia" occurred 24 hours after the operation and persisted. A study of the stained films of the blood of both monkeys revealed such signs of regeneration as nucleated red blood cells, polychromatophilia, basophilic stippling and an increase in the number of reticulocytes. This response is similar to that seen in "secondary anemias" in man.

Summary. Following total gastrectomy in monkeys, a "secondary" type of anemia occurred 72 hours after operation, and persisted. Evidence of regeneration (reticulocytosis, nucleated red blood cells, polychromatophilia and basophilic stippling) was noted. The specific blood picture of human pernicious anemia did not develop within the 6 month period of study, although there was a decrease in the number of red blood cells, a slight tendency for the color index to rise above the preoperative value, a leukopenia and a slightly increased degree in size dispersion of the red blood cells.

7659 P

Effect of Calcium and Phosphorus in Diet of Mothers upon Weight of Young.

WARREN M. COX, JR., AND MIRIAM IMBODEN. (Introduced by C. E. Bills.)

From the Research Laboratory, Mead Johnson and Company, Evansville, Indiana.

In spite of an immense amount of work on the *retention* of calcium and phosphorus under various conditions the amount or ratio of these elements recommended for pregnant or lactating animals is still almost empirical. Before dependable knowledge of the optimal ratio and amounts of these elements during such periods can be obtained numerous animals should be studied through their whole reproductive life. In order to test thoroughly any one calcium and phosphorus mixture it would be desirable (1) to secure as *rapid reproduction* as possible, and (2) to provide constant quantities of these elements during the animal's entire reproductive history. We have made such a study during the past 2 years, using female rats, and report in this preliminary communication one of our more important findings.

Method. Virgin female rats weighing approximately 200 gm. at 100 days of age, were allowed to raise their first, "qualifying" litter on stock food. The females were remated at the end of 21 days' lactation and placed on the experimental diet.* All litters were reduced to 6 pups. When the young were 21 days old they were killed, and the mother immediately remated—thus allowing no rest period between cycles. In this way 10 successive gestations and lactations were studied. Five females were placed on each mineral combination, and the average "success" of the 5 mothers was used as a criterion of the adequacy of the calcium and phosphorus mixture. The "success" of the rats was gauged by averaging, over 10 reproductive cycles, the weight of the young at 21 days of age.

Salt Mixtures. Each of 5 levels of calcium was combined with 5 levels of phosphorus to give 25 experimental diets, with Ca/P ratios from 0.1 to 10.0. This is best presented graphically,

* Acid-washed casein 20%; dextrin 50.1-55.1%; lard 9%; yeast concentrate 4%; wheat germ oil 1.6%; carotene 3:1000, 0.3%; salts (Ca and P free) 3.1%; rice cellulose 1.2-5.9%; Ca and P salt mixture 1.0-10.7%. No vitamin D.

These percentages were obtained by mixing suitable quantities of $CaHPO_4$, $Ca(CH_3COO)_2$, and $(NH_4)H_2PO_4$, and incorporating in the standard diet.

Results. In the course of this experiment 3,431 rats have been raised to 21 days of age. The average weight of the rats *actually raised* on each diet is charted in Fig. 1 against the Ca/P ratio of

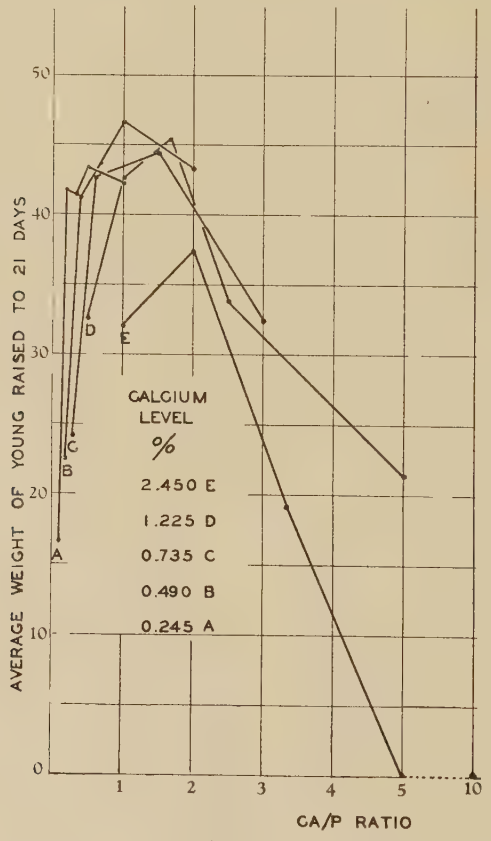


Fig. 1.

Average weight of total number of young raised by mothers on each experimental diet plotted against the Ca/P ratio of the diet. A, B, etc., refer to the percentage calcium in the diet.

the diet. There are 5 curves, each corresponding to a given level of calcium, and (from right to left along the curve) increasing levels of phosphorus. Viewed as a whole, 4 of the 5 curves presented in Fig. 1 permit the construction of an average curve that would indicate maximum performance at, or very near to, a Ca/P ratio of 1.0.

However, when the individual curves are examined it is seen that at the *0.245% calcium level the largest rats were raised at a Ca/P ratio of 0.5. When the calcium level was 0.490%, the Ca/P ratio at which the largest rats were raised, was 1.0.* The results of such examination can be tabulated as follows:

% Ca in diet	Largest Young Raised at Ca/P ratio of	Average wt. at 21 days
0.245	0.50	43.3
0.490	1.00	46.7
0.735	1.50	44.4
1.225	1.66	45.4
2.450	2.00	37.3

It is, we believe, fair to consider the average weight of the young raised during 10 reproductive cycles as a criterion for the suitability of the Ca/P ratio of the diet. As stated previously, if an *average* curve for the 4 lowest levels of calcium were constructed, the optimum ratio would be at, or very near to, 1.0, but inasmuch as *each* level of calcium used showed a maximum weight of young at a different Ca/P ratio we must conclude that no one optimum ratio can be stated unless at the same time the amount of calcium is known. The level of calcium in a diet, therefore, determines what level of phosphorus, *i. e.*, what Ca/P ratio, is optimal.

7660 C

Nature of the Blood-Cerebrospinal Fluid Barrier Permeability Revealed by Isohemagglutinin Tests.

LELAND W. PARR AND RAYMOND H. GOODALE.

From the Department of Bacteriology, Hygiene and Preventive Medicine, School of Medicine, The George Washington University, Washington, D. C., and the Laboratory Department, City Hospital, Worcester, Mass.

It has been stated that blood-cerebrospinal fluid barrier permeability is capricious. This is possibly a confession that the meaning and mechanism of permeability are yet incompletely understood. It is not surprising that this is so for careful study of standard texts and original papers since 1910 leads one to conclude the theories of origin, function and fate of cerebrospinal fluid are far from agreed upon.

Studies of variations in the permeability of the barrier have been prosecuted from two points of view. Foreign substances have been introduced, more commonly into the blood stream, occasionally into the subarachnoidal space, and their fate traced. Such substances have been crystalloids, as bromide; colloids, colored and uncolored, and McKinley and Holden[1] utilized the bacteriophage. Again, study has been made of blood elements not normally found in the cerebrospinal fluid whose presence there may be taken as evidence of increased permeability. Of these may be mentioned induced antibodies and naturally occurring antibodies such as hemolysins and isohemagglutinins. Three references exist dealing with this last indicator of permeability.

Weil and Wall[2] found no isohemagglutinins in 12 cerebrospinal fluids from such conditions as syphilis of the central nervous system, hemiplegia, brain tumor and myoclonic encephalitis. On the other hand Herman and Halber[3] found isohemagglutinins in 11 of 80 fluids examined. These fluids came from more than 30 widely different types of pathology, although most involved the central nervous system either organically or functionally. In 6 of 22 cases of bacterial meningitis the cerebrospinal fluids contained isohemagglutinins but the 6 cases of tuberculous meningitis included were all negative. Two of 3 cases of cord tumor, 2 of 4 cases of sclerosis, and 1 of 3 cases of chorea accounted for the remaining 5 positives. Parr[4] examined 104 cerebrospinal fluids at Beirut and found 6 containing isohemagglutinins.

These suggestive but far from conclusive results led the present authors to further investigate the problem. The cerebrospinal fluids examined came from the services of the Worcester City Hospital and represent material from cases of all ages and both sexes. They were forwarded to Washington for examination. All found colored, contaminated, or otherwise unfit for use were discarded. Tests were carried out on 156 different cerebrospinal fluids by mixing small quantities of the fluid under test with equal volumes of 5% saline suspensions of (1) known type A red blood cells, and (2) known type B red blood cells. The mixtures were sealed in well slides by means of vaselined coverslips and incubated from one to 2 hours. The preparations were then uncovered, agitated and ex-

[1] McKinley, E. B., and Holden, M., Proc. Soc. Exp. Biol. and Med., 1927, **24**, 595.

[2] Weil, P. E., and Wall, P. I., *Compt. Rend. Soc. Biol.*, 1923, **88**, 173.

[3] Herman, E., and Halber, W., *Compt. Rend. Soc. Biol.*, 1924, **91**, 959.

[4] Parr, L. W., *J. Lab. and Clin. Med.*, 1932, **17**, 333.

amined, readings being made as one would make them in typing unknown blood sera by the use of known red blood cells. The patients from whom the fluids were taken were blood typed in Worcester and these data together with clinical and pathological information were made a part of the record.*

It should be recalled that persons of blood type AB (Jansky 4) have no isohemagglutinins in their blood and in such cases one would not find isohemagglutinins in the cerebrospinal fluid even with a highly permeable barrier. Such persons, however, number less than 5% of our population and do not materially complicate the picture.

In 21 of the 156 specimens typed isohemagglutinins were found. These positive specimens were from cases of skull fracture, multiple sclerosis, lues, cerebral hemorrhage, post-operative and post-partum symptomatology, epilepsy, pneumonia, sinus infection, bronchitis, influenzal meningitis and concussion. Negatives derived from these same conditions and also from poliomyelitis, encephalitis, uremia, brain tumor, tuberculous meningitis, hypertension, pulmonary tuberculosis, anemia, senile dementia, psychoneurosis, convulsions, hydrocephalus, alcoholism, diabetes, mastoiditis, Korsakoff's syndrome, endometritis, Adams-Stokes syndrome, myelitis, pyelitis, influenza, nephritis, paresis, tabes dorsalis, rheumatism, gas and also barbital poisoning, neoplasm, cholecystitis, general trauma, and amebic dysentery.

Our experience corroborates the impression previously held that such permeability of the blood-cerebrospinal fluid barrier may exist as to permit passage of isohemagglutinins from the blood. However, we have not been able to demonstrate that the passage of these natural antibodies occurs with constancy in any given pathological condition, nor were the positives obtained correlated with age, sex, or racial stock.

The test for isohemagglutinins in the cerebrospinal fluid is slow and fresh red blood cells of 2 types are needed to carry it out. It seems unlikely that such a procedure has much practical value either in the understanding of permeability *per se* or in clinical laboratory diagnosis. Lastly, from the point of view of the occurrence of isohemagglutinins in cerebrospinal fluid barrier permeability is capricious.

* For valuable assistance at Worcester we are indebted to Mrs. Dorothy Kiniry.

7661 P

Amino Acids Required by the Diphtheria Bacillus for Growth.

J. HOWARD MUELLER.

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Mass.

The writer[1] described experiments indicating that a strain of diphtheria bacillus under investigation required for its growth, among other things, a substance associated with the "proline" fraction of amino acids obtained by Dakin's butyl alcohol extraction method. Further work with this material has shown that not a single factor, but 3 separate substances were involved in the accelerating effect there described. It was learned to begin with, that the action was not entirely limited to the "proline" fraction, but that the "monoamino" fraction even more frequently exerted it, and occasionally the 2 added together gave better results.

Before any material progress was made in separating and recognizing these factors, it became necessary to introduce a quantitative method for the estimation of relative amounts of growth. The method finally used was a micro kjeldahl determination of the amount of nitrogen in the bacterial growth on a measured amount of material for a definite time. The details of this procedure will be described elsewhere.

Careful examination of the effect produced by the proline fraction showed that proline itself was evidently not concerned, but that the acceleration in growth was apparently produced by minute quantities of ethyl alcohol used as solvent in handling the fraction. Pure ethyl alcohol exerted the same effect, and maximum acceleration of growth with our particular strain of organism is reached with a concentration of about 0.5%. Larger quantities of course become inhibitory. In place of ethyl alcohol, sodium acetate, glycerol, or maltose all appear to enhance the growth when added to the same control solutions.

The monoamino fraction, presumably containing glycine, alanine, valine, leucine, oxyproline, phenylalanine, tyrosine and methionine was next examined. Of these substances, only methionine showed any material acceleration of growth, when added to suitable control solutions. Its effect is striking in that small quantities, 0.25 to 0.75 mg. in 10 cc. of media approximately treble the growth on the con-

[1] Mueller, J. H., Klise, K. S., Porter, E. F., and Graybiel, A., *J. Bact.*, 1933, **25**, 509.

trol, whereas with slightly larger quantities, 2.0 to 3.0 mg., the growth begins to decrease and drops progressively as the amount of methionine is increased, so that with amounts of between 10 and 40 mg. in 10 cc., the growth is but little better than, but appears not to drop below that which occurs in the control.

Addition of the crude "monoamino" fraction to media containing sufficient methionine to throw the curve on the downward slope, which of course introduces still more methionine, will nevertheless sharply increase the growth obtained to about twice the maximum obtained with methionine alone. None of the other recognized constituents of the mixture, either alone or in combination will produce a similar effect. Attempts at chemical isolation of this third factor supplied the information that it was precipitable with Ag_2O and $Ba(OH)_2$, and trial showed that histidine, which is not supposed to be present in the fraction under investigation, would practically duplicate the effect of the crude mixture. The explanation for the seemingly combined effect of methionine and histidine is not apparent, and may prove to be of interest in connection with methionine metabolism in the animal body.

In the meantime it was found that glutamic acid also increased markedly the amount of growth obtained in controls containing the crude monoamino fraction. The effect is not shared by aspartic acid nor asparagine under the conditions of the experiment, and the action differs from that of the other amino acids now shown to be necessary in that in each other case, maximum growth is reached with a concentration of about 1.0 mg. per 10 cc. of medium, while with glutamic acid the effect is slowly progressive, and the curve of growth still ascends gradually with 50 or 60 mg.

The medium as at present prepared, which gives maximal growth with our strain has the following composition:

	%
Liebig's extract	.75
Salt mixture*	
Cystine	.01
Tryptophane	.01
dl or l Methionine	.01
Histidine hydrochloride	.01
Glutamic acid hydrochloride	.5
Ethyl alcohol	.5
Phenol red	.001

*The salt mixture now used is prepared as follows:

NaCl	70.0 gm.
K_2HPO_4	2.5
$CaCl_2$	0.15
$MgSO_4$	0.15
$FeCl_3$	0.05
HCl (conc.)	0.5 cc.
H_2O to 350 cc.	

0.25 cc. of this solution is added to each 10 cc. of media.

The reaction is brought to pH 7.4-7.6 by addition of NaOH, the phenol red in the medium serving as the indicator, and autoclaved at 10 lb. for 10 minutes. Each tube is inoculated with a 3 mm. loopful of pellicle from a 24-hour broth culture and placed in a slanted position in the incubator.

On 10 cc. of such media approximately 1.9 mg. bacterial nitrogen is formed in 55-60 hours at 35°. The omission of either cystine, tryptophane or methionine causes a drop in nitrogen to the level of 0.2 to 0.3 mg. The growth obtained with this same strain on the usual meat infusion-peptone broth (10 cc.) gives 0.8-0.9 mg. nitrogen.

7662 C

A Renal Lesion Occurring in Rats Maintained at Low Environmental Temperatures.

EATON M. MACKAY, ERNEST M. HALL, AND FRANCIS M. SMITH.

From the Scripps Metabolic Clinic, La Jolla, California, and the Department of Pathology, University of Southern California, School of Medicine, Los Angeles.

During a study of the influence of environmental temperature upon the metabolic rate and certain organ weights of the albino rat a number of these animals were maintained at room temperature of 5° and 10°C. When they were killed it was noticed that the kidneys of many were swollen, soft and mottled in appearance. Fig 1. His-

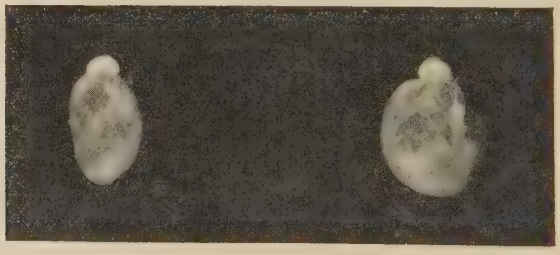


FIG. 1. Gross appearance of kidneys from rats Nos. 6 and 99, grown at 5° C.

tological examination revealed an interesting lesion in their kidneys. Besides its inherent interest it seemed desirable to report the occurrence of this lesion because of the common belief that exposure to cold is often associated with the onset of one form of Bright's disease in man. That the renal lesion reported here was acutally due to the cold environment seems certain for only 0.015% of the

kidneys from 26,000 animals of the same stock raised at ordinary temperatures have shown any renal pathology.

Eighteen young adult rats, 9 males and 9 females were kept 3 in a cage at a constant environmental temperature of 5°C. for 90 days. By this time 7 were dead. Two of these were examined and both had mottled soft kidneys. Of the 11 which remained 4 showed the presence of the renal lesion grossly. Twenty-four young adult rats, 12 males and 12 females were kept under the same conditions as the first group at a temperature of 10°C. for 90 days. When they were killed 6 showed quite obviously the renal lesion to be described here.

Microscopic examination reveals depressed areas in the superficial part of the cortex which appear to be due to partial collapse of the tubules in these areas with necrosis and desquamation of the epithelial cells. Some of the cells are shrunken and clumped together so that they appear as partly fused cellular masses in which the nuclei are very hyperchromatic and closely crowded. The cytoplasm tends to stain a muddy pink or smudgy blue (H. & E.). Masses of amorphous or finely granular calcareous deposits which stain deeply with haematoxylin are found among the desquamated cells, Fig. 2. In the collecting tubules of the medulla granular, dark blue staining casts are found in moderate numbers. These apparently contain calcium.

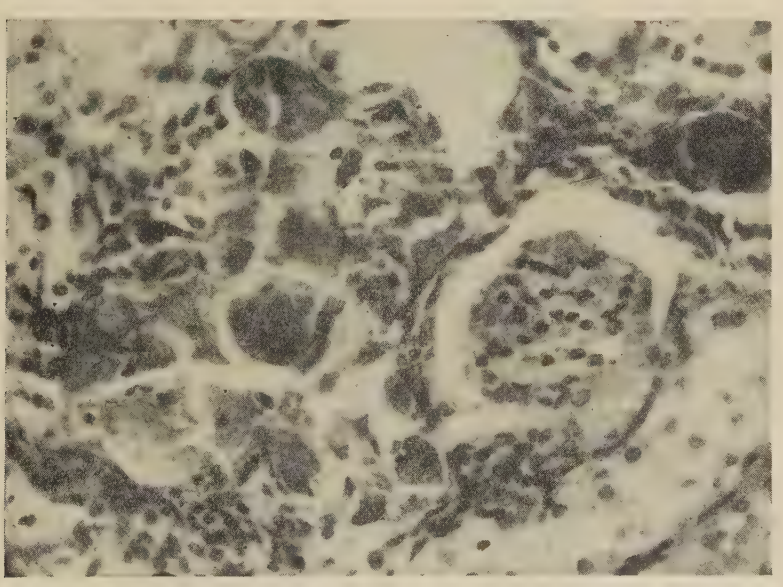


FIG. 2. Necrosis of tubular epithelium with precipitation of calcium. Note the dark-staining mass in the upper right hand corner.

Between the depressed areas may be seen tubules filled with swollen, granular epithelial cells which stain quite intensely with eosin. The lumina of the tubules are practically obliterated by the swollen cells. The nuclei are pycnotic in many of the cells and the cytoplasm shows radial fissuring. Throughout the cortex are groups of "ghost" tubules which belong largely to the proximal convoluted portions of the tubular units. In these the nuclei have disappeared completely or show but faintly while the cytoplasm is granular and pale-staining, Fig. 3. In some of the tubules irregular hyaline masses are seen. In some areas whorls and strands of this material fill tubules in a bizarre manner. The tubules everywhere seem to be moderately to severely damaged.

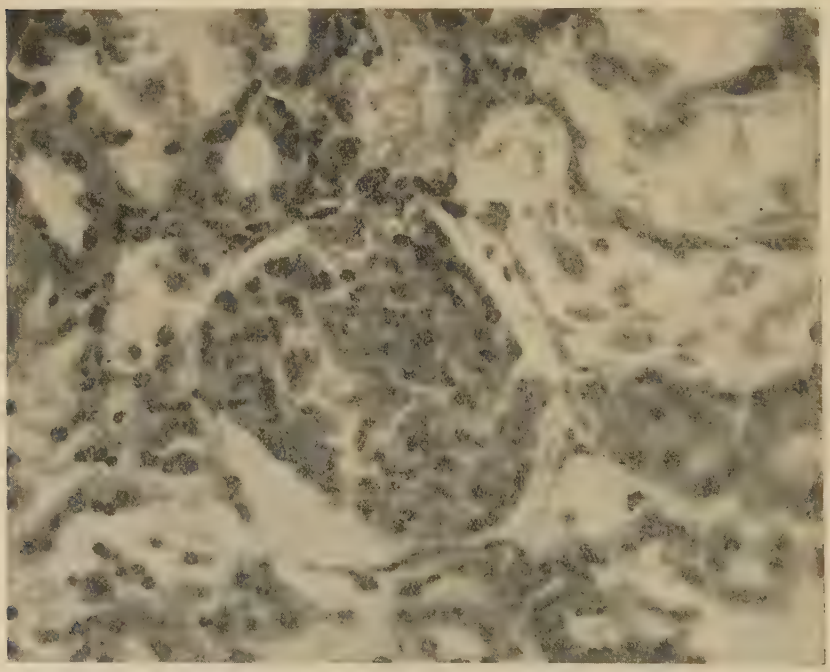


FIG. 3. Glomerulus under high power showing agglutination of red blood cells within the capillary loops. Several of the proximal convoluted tubules show almost complete necrosis of their epithelium.

The glomeruli are greatly congested. The capillary loops are moderately dilated and filled with clumps of red cells which are partially or completely fused into hyaline masses, Fig. 3. Not only is this true in the glomeruli but the red cells in the smaller and larger vessels show a marked tendency to agglutination, Fig. 4. The severe changes described above are seen in the kidneys of rats grown at

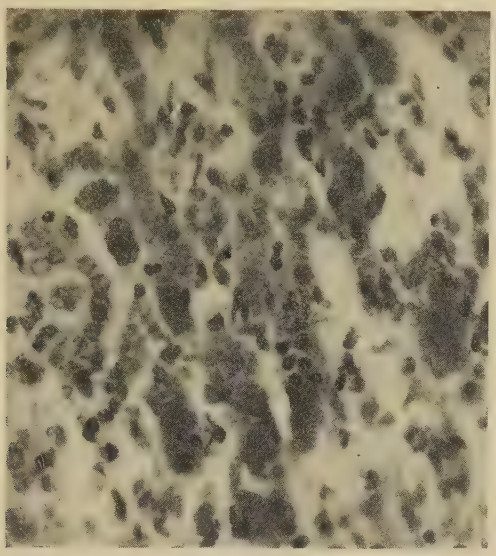


FIG. 4. Marked clumping of red cells in the capillaries of the medulla.

5°C. A larger series, 7 female and 2 males grown at 10°C. all show precisely similar changes varying only in the degree of severity.

The mechanism which leads to the development of tubular necrosis is not clear. The presence of agglutinated and hyalinized red corpuscles in the vascular channels and in the glomeruli suggests at once the immediate cause. All of the 13 animals examined histologically reveal this hemagglutination with (probably ensuing) necrosis of the tubular epithelium. The deposition of calcium in some of the areas is no doubt secondary to the necrosis. As for the cause of the initial hemagglutination we have no evidence to offer at this time.

7663 C

Rate of Absorption of Glucose from the Intestinal Tract of the Rabbit.

EATON M. MACKAY, H. C. BERGMAN AND RICHARD H. BARNES.

From the Scripps Metabolic Clinic, La Jolla, California.

We concluded elsewhere[1] that the true relation of the rate of absorption of glucose from the intestine to the concentration in this

[1] MacKay, E. M., and Bergman, H. C., *J. Biol. Chem.*, 1933, **101**, 453.

TABLE I.

Rate of Absorption of Glucose from the Intestinal Tract of Rabbit. Each Figure is an Average of a Group of 3 Rabbits.

Hr.	Aver. Body Wt.	Dose of Glucose	Glucose in Stomach at end of period	Conc. Glucose in Stomach	Glucose in Intestine at end of period	Aver. amt. glucose available for absorption during period	Glucose absorbed during period	Aver. glucose absorption per hour of period	Approx. amt. of glucose absorbed each hour	Approx. amt. glucose available for absorption each hour
	gm.	gm.	gm.	%	gm.	gm.	gm.	gm.	gm.	gm.
1	1670	8.85	4.16	7.06	2.08	4.69	2.61	2.61	2.61	4.69
2	1630	8.15	2.81	4.82	1.64	5.34	3.70	1.85	1.09	2.73
3	1690	8.45	2.21	4.24	1.90	6.24	4.34	1.44	0.64	2.54
4	1630	8.15	2.03	3.26	1.12	6.12	5.00	1.25	0.66	1.78
5	1605	8.02	0.87	2.02	0.84	7.15	6.31	1.26	1.31	2.15
6	1600	8.00	0.11	0.35	0.67	7.89	7.22	1.20	0.89	1.58

organ can only be established in experiments in which the stomach contents are considered separately. Attempts to carry out such experiments on rabbits were partially unsuccessful for no reasonable period of starving will free the gastrointestinal tract of debris and these animals do not tolerate starvation well unless roughage is supplied. One finding was of interest, namely, support of our contention[1] that the rate of glucose absorption per unit time from the intestinal tract of rats is not constant but dependent on the amount and concentration of glucose administered and always decreases with time after the sugar is given. The Coris[2] and others[3] have presented polemical evidence against this view.

A typical experiment is presented in Table I. Each figure is an average of a group of 3 albino male rabbits of approximately the same age and weight. Each animal received 30 cc. of 5% agar-agar per day during a 3-day starvation period preceding the experiment. To estimate the glucose absorption Cori's method[4] was used with the modification that the stomach contents were examined separately and the concentration of the glucose as well as the amount in this organ determined. The rabbits were given 10 cc. per kilo body weight of a 50% glucose solution. They were killed by air embolism, air being injected into the ear vein. When killed the stomach and intestine contained considerable swollen agar-agar. This vitiated the importance of concentration changes. It is interesting, however, that within even a single hour the glucose concentration in the stomach becomes so greatly reduced. Ravdin, Johnston and Morrison[5] have noted this tendency for dilution to a rather uniform figure.

Our doses of glucose were given in relation to body weight. In the rat the rate of absorption is more dependent on body surface than body weight.[1] This is also probably true for the rabbit but since no reliable body surface factor was available for this species and all of our animals weighed very nearly the same we have used body weight. Since the average weights of all the groups were for practical purposes identical the results have simply been expressed per rabbit.

Maddock *et al.*[3] have definitely demonstrated that no glucose is absorbed from the stomach. Consequently we have treated this

2 Cori, C. F., and Cori, G. T. *Ann. Rev. Biochem.*, 1933, **111**, 151. Stanford University Press.

3 Maddock, S. J., Trimble, H. C., and Carey, B. W., Jr., *J. Biol. Chem.*, 1933, **103**, 285.

4 Cori, C. F., *J. Biol. Chem.*, 1925, **66**, 691.

5 Ravdin, I. S., Johnston, C. G., and Morrison, P. J., *Am. J. Physiol.*, 1933, **104**, 700.

organ as a reservoir. Our results show very clearly from the average rate of absorption per hour for the various periods that the rate of glucose absorption is greatest during the first hour after giving the glucose and decreases each hour thereafter. For at least the first 4 hours after administering the glucose this fall in the absorption rate cannot be due to any lack of glucose for absorption. Since there is no absorption from the stomach the amount of glucose available for absorption each hour is that which reaches the intestine plus any unabsorbed during the preceding hours. There is a tendency for a relation in a general way between the absorption rate and the amount of glucose available for absorption. That this is not more clear-cut may be due to the fact that not only is the amount but also the concentration of glucose leaving the stomach becoming less each hour.

These results in so far as they are comparable confirm similar experiments on the rat.[1] The results in both species coincide well in many aspects with the experiments of Ravdin *et al.*[5] on the rate of glucose absorption from isolated intestinal loops in the dog.

Summary. The rate of absorption of glucose from the intestinal tract of the rabbit decreases with time after the glucose is administered. This is apparently related to the decreasing amount of glucose entering the intestine from the stomach each hour for there is a relation between the rate and amount of glucose available for absorption.

7664 P

Observations upon Hypophysectomized-Depancreatized Cats.

C. N. H. LONG AND F. D. W. LUKENS.

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

Houssay and Biasotti,[1] Barnes and Regan[2] and others have demonstrated the prolongation of life and amelioration of the diabetes that follows total pancreatectomy in the hypophysectomized dog. During the last few months observations have been made upon 2 cats in which total pancreatectomy was performed 2 weeks after hypophysectomy. There was no loss of weight in the period be-

1 Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511.

2 Barnes, B. O., and Regan, G. F., *Endocrinology*, 1933, **17**, 522.

tween the 2 operations. One animal died in hypoglycemia on the 35th day after pancreatectomy, the other was killed on the 85th day while still in good health for liver glycogen determination (0.8 g %). Neither of these animals received insulin at any time. Both of the animals lost almost 50% of their body weight during the periods noted. They were somewhat apathetic in their behavior and showed considerable muscular flaccidity although capable of ordinary activity with no apparent distress. Their appetite was capricious but their food intake was usually ample for the caloric requirements of normal animals.

In 9 control animals the average length of life after pancreatectomy or withdrawal of insulin was 4 days (range 2-7 days). The glycosuria averaged 5-10 gm. daily in spite of the fact that the animals usually refused food after the second day.

Cat I (lived 35 days) had had the right adrenal removed prior to the hypophysectomy. After total pancreatectomy it consumed 50-150 gm. of raw pancreas and liver daily. The glycosuria was about 2 gm. daily with occasional sugar free days. The maximum glucose excretion at any time was 4.8 gm. No ketonuria was noted (Rothera's test), although observations upon this were only made at infrequent intervals. The fasting blood sugar ranged from 59-314 mg. % while the terminal blood sugar was 15 mg. %, the urea N 32 mg. % and the plasma CO_2 56 vol. %.

On the 17th day of life the animal developed a subcutaneous abscess which burst spontaneously and healed. No exaggeration of the diabetic condition developed during the course of this infection.

At autopsy no pituitary or pancreatic tissue was found and histological examination of the testes, thyroid and remaining adrenal revealed the characteristic changes associated with hypophysectomy.

Cat II (survived 85 days) ate larger quantities of food than Cat I and showed a much greater degree of glycosuria ranging from 2-17 gm. daily. The daily urine volumes were 150-200 cc. in contrast to Cat I which averaged about 60 cc. A few observations on ketonuria were negative. As this cat was observed over the summer vacation only 8 determinations were made on the fasting blood sugar. They ranged from 225-302 mg. %.

As stated above, this animal was sacrificed for glycogen determinations. At autopsy a small piece of pituitary, chiefly posterior lobe, was found. Such anterior lobe tissue that was present was undergoing degenerative changes but nevertheless may have been responsible for the differences between the animals. The usual changes were observed in the ovary, thyroid and adrenal.

The first point of these experiments is the extension of the effects of hypophysectomy upon experimental diabetes to another species.

The second point is the prolongation of life of depancreatized cats, a species in which this operation alone is so uniformly and rapidly fatal. These control animals developed severe acidosis and ketosis about 48-72 hours after removal of the pancreas. In both of the doubly operated animals, so far as our observations go at present, neither acidosis nor ketosis was a prominent feature. The other characteristics of diabetes—glycosuria, polyuria and marked loss of weight, were present in varying degree.

7665 C

A Note on the Relationship of Pellagra to Pernicious Anemia.

TOM D. SPIES, WARREN PAYNE, AND AUSTIN B. CHINN. (Introduced by J. T. Wearn.)

From the H. K. Cushing Laboratory of Experimental Medicine, Department of Medicine, Western Reserve University, and the Medical Services, Lakeside Hospital and the Cleveland City Hospital, Cleveland.

Pellagra and pernicious anemia are now regarded as special types of deficiency diseases. They have in common such clinical manifestations as achylia gastrica, glossitis, peripheral neuritis, and central nervous system changes. Goldberger and his associates[1, 2] believe that pellagra is caused solely by a diminished intake of some specific food substance ("vitamin G"). Rolph,[3] Turner,[4] and others have described cases developing secondary to lesions of the gastro-intestinal tract. While it has likewise been suggested that in certain instances the lack of some substance in the diet may cause pernicious anemia, it usually follows the lack of the essential secretion ("intrinsic factor") in the gastric juice which changes food into an anti-anemic substance.[5] A short time ago Spies and Payne[6] produced remissions in 2 patients with pernicious anemia, by giving an incubated mixture of beef muscle and achylic gastric juice from acute

1 Goldberger, J., and Wheeler, G., Bull. 120, Hygienic Laboratory, Washington, 1930, 120.

2 Goldberger, J., Wheeler, G., Lillie, R. D., and Rogers, L. M., *U. S. Public Health Report*, 1926, **41**, 297.

3 Rolph, F. W., *Canad. M. A. J.*, 1916, **6**, 323.

4 Turner, R. H., *Am. J. Trop. Med.*, 1929, **9**, 192.

5 Castle, W. B., Health, C. W., Strauss, M. B., *Am. J. Med. Sci.*, 1931, **182**, 741.

6 Spies, T. D., and Payne, W., *J. Clinical Invest.*, 1933, **12**, 229.

pellagrins. This observation indicated that the "intrinsic factor" was present in gastric secretions from pellagrins in an amount adequate to form the antianemic substance. They suggested in this study that the usual pellagrin apparently develops his disease as a result of inadequate food ingestion, whereas the usual pernicious anemia patient developed the anemia from the failure of his gastric juice to make an antianemic substance from food.

Since pernicious anemia and pellagra are both related to diet and its subsequent assimilation by the body, there have been recent attempts to recognize more specifically those substances in food which are important in the pathogenesis of the 2 diseases. Strauss and Castle[7, 8] have found that the substance in food ("extrinsic factor") concerned with the development of pernicious anemia is associated with so-called "vitamin G" which other workers have considered as the "antipellagric vitamin". Wills[9] has observed that this vitamin is not identical with the substance curing tropical macrocytic anemia, and was unable to obtain a satisfactory hemopoietic response from the vitamin B complex (including vitamin G).

In view of the close relationship between pellagra and pernicious anemia, and the known fact that under certain conditions yeast has been curative for both, we have studied the relative efficacy of autoclaved brewer's yeast* in the 2 diseases during the past 3 years.

Five cases of classical Addisonian pernicious anemia and 30 cases of characteristic pellagra were selected. Patients with pernicious anemia were given ordinary hospital diets without liver, kidney or pancreas. After a preliminary period of observation, each of 3 cases was given daily an incubated mixture of 150 cc. of normal human gastric juice and 50 gm. of autoclaved brewer's yeast. (This yeast was autoclaved by the Harris Laboratories and shipped in sealed 5 lb. tins). The 2 other patients with pernicious anemia were given the same amount of yeast after it had been incubated with pepsin and trypsin instead of human gastric juice. The course of the anemia in the 5 cases was followed by daily determinations of red blood cells, hemoglobin, and reticulocytes but no significant change occurred during the 10 days which they received the incubated materials. Each patient with pernicious anemia responded promptly to the administration of intramuscular liver extract which was given immediately following the experimental period.

7 Strauss, M. B., and Castle, W. B., *New Eng. J. Med.*, 1932, **207**, 55.

8 Strauss, M. B., and Castle, W. B., *Lancet*, 1932, **2**, 111.

9 Wills, L., *Lancet*, 1933, **1**, 1286.

*Furnished through the courtesy of Dr. Isaac F. Harris, Tuckahoe, N. Y.

The pellagrins were given a diet low in pellagra-preventive substances throughout the course of the experiment and autoclaved yeast from the same tin which contained the dry powder given to the pernicious anemia patients was administered to each pellagrin in daily quantities of 50 to 100 gm. / All signs and symptoms of pellagra promptly disappeared under controlled conditions, indicating that the yeast contained a chemical substance which could be utilized by the pellagrous patient. On the other hand, the failure of a hemopoietic response to the incubated mixture of yeast and gastric juice in those patients with pernicious anemia suggests that either an antianemic substance was not formed in adequate amounts to remit the disease under the condition of this experiment, or, if formed, the antianemic factor was not utilized by the patient. / The digestion of the same preparation of yeast by means of pepsin and trypsin likewise produced no substance which remitted the patient.

Strauss and Castle[7, 8] produced a hemopoietic response in pernicious anemia patients by giving them an incubated mixture of normal gastric juice and *Vegex,* a preparation of autolyzed yeast and "flavoring materials". On theoretical grounds it seems likely that the process of autolysis of yeast which *Vegex* undergoes might cause many physical and chemical changes in the yeast cells which might not be produced by autoclaving under steam pressure for long periods of time as was done with the brand of yeast we used. (Lassen and Lassen[10] have just published some observations showing that yeast mixed with human gastric juice does not produce a hemopoietic response in pernicious anemia patients.) It is conceivable that the autoclaving process may firmly bind potent substances so that they cannot be utilized and that autolysis may break down the yeast cells and liberate active substances. The quantitative relationship between the amounts of gastric juice and any available precursor of the antianemic substance ("extrinsic factor") may have been insufficient to produce a satisfactory hemopoietic response in the anemia patients during 10 days and for that reason, our observations should not be interpreted as demonstrating that the autoclaved brewer's yeast does not contain a substance which under certain circumstances might be activated and changed into an antianemic factor. The pellagrins, on the other hand, did utilize the same preparation of yeast for the cure of this disease. / The present study suggests that the chemical substance in yeast, utilized by the pellagrin to remit his disease, is not the same as the precursor of the antianemic factor found in food ("extrinsic factor").

[10] Lassen, H. C. A., and Lassen, H. K., *Am. J. Med. Sci.,* 1934, **188**, 461.

7666 C

Effect of Salt Treatment on Certain Changes Following Adrenalectomy.

R. L. KUTZ, T. McKEOWN, AND H. SELYE. (Introduced by J. B. Collip.)

From the Department of Biochemistry, McGill University, Montreal.

It has been shown[1, 2, 3] that the loss of sodium chloride and possibly other inorganic ions contributes to the development of the severe symptoms which follow adrenalectomy, and that the administration of these ions will partially or completely prevent the development of these symptoms.

Since disturbances of the oestrous cycle have repeatedly been studied in adrenalectomized animals,[4, 5, 6] and since various authors came to the conclusion that cortical extracts have a specific influence on the sex organs,[6, 7, 8] it seemed of importance to establish whether the administration of salt would correct the disturbance of the cycle, as our preliminary experiments seemed to indicate.[9]

Forty-six normally cyclic rats were adrenalectomized. Fifteen of these were untreated, and went into a dioestrus which lasted until death. The remaining 31 were given the Rubin salt mixture.[3] Of the 18 animals maintained in good health by salt, 14 remained normally oestric; the animals which died in spite of treatment were nearly all dioestric until death. The completeness of the operation was assured by the death which followed soon after the removal of the salt, and by the absence of any cortical tissue at autopsy.

In general, then, it seems to be true that where the salt mixture maintains the life and weight of the adrenalectomized animal, oestrus appears at normal intervals. In this connection we would like to emphasize that the salt mixture maintained only 18 of the 31 animals treated.

---

1 Loeb, R. F., Atchley, D. W., Benedict, E. M., and Leland, J., *J. Exp. Med.*, 1933, **57**, 775.

2 Harrop, G. A., Soffer, A. J., Ellsworth, R., and Trescher, J. H., *J. Exp. Med.*, 1933, **58**, 1.

3 Rubin, M. I., and Krick, E. T., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 228.

4 Martin, S. J., *Am. J. Physiol.*, 1932, **100**, 180.

5 Wyman, L. C., *Am. J. Physiol.*, 1928, **86**, 528.

6 Corey, E. L., and Britton, S. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 592.

7 Corey, E. L., *Am. J. Physiol.*, 1933, **105**, 24.

8 Migliavacca, A., *Fol. Gynaec.* (Genova), 1932, **29**, 453.

9 Kutz, R. L., McKeown, T., and Selye, H., *Royal Society of Canada*, May, 1934.

As an incidental finding we observed that unusually large quantities of a greenish yellow pigment, giving the Prussian Blue reaction, were deposited in the spleens of all adrenalectomized animals, treated or untreated. Similar depositions have also been observed in the spleens of hypophysectomized animals. The decrease in number of eosinophiles in the hypophysis which usually follows adrenalectomy was not influenced by the salt treatment.

From these experiments we conclude that the dioestrus following removal of the adrenals is probably not the result of the absence of any specific sex hormone found in the adrenal glands, but rather of the general ill-health of the animal.

7667 P

Oxidation of Cystine Sulfur to Sulfate by Ferric Chloride.

ROBERT H. SIFFERD AND VINCENT DU VIGNEAUD.

From the Department of Biochemistry, School of Medicine, George Washington University, Washington.

The oxidation of thiol-imidazole sulfur to sulfate by $FeCl_3$ is frequently used in the conversion of thiol-imidazole compounds to imidazoles. One would assume from the literature that cystine sulfur is not similarly oxidized by this reagent.[1] This suggested the utilization of this reaction as a means of testing for the possible presence of a thiol-imidazole compound in insulin. So far only about 70% of the sulfur of insulin has been accounted for. The highly specific Sullivan reaction indicates the presence of about 8.4% cystine, whereas if all the sulfur of insulin (3.2%) were present as cystine, 12.0% should be present.

To test the feasibility of this approach experiments with cystine were first undertaken. These experiments showed contrary to our expectations that cystine sulfur was quite readily oxidized to sulfate by $FeCl_3$. Twenty mg. of cystine heated with 2.5 cc. of 6.5% $FeCl_3$ for 1 hour in the boiling water bath gave a positive test for sulfate. Under the same conditions no sulfate was formed from cysteic acid.

Other experiments on cystine were conducted in which the compound was heated in $FeCl_3$ (6.5%) with HCl varying in concentration from N/10 to 10%. All samples showed appreciable amounts of sulfate after being heated for an hour. The amount of oxidation

[1] Barger, G., and Ewins, A. J., *J. Chem. Soc.*, 1911, **99**, 2336.

decreased in a roughly proportional manner with the increase in acid content, yet we found no conditions under which no sulfate was obtained.

To determine the amount of sulfate formed a 5 gm. sample of *l*-cystine was suspended in 625 cc. of a 6.5% $FeCl_3$ solution and was heated on a boiling water bath for a total of 21 hours. Samples were removed at intervals for sulfate determination. The oxidation followed roughly an exponential curve of low coefficient. At 21 hours approximately 60% of the sulfur was accounted for as sulfate.

The failure of cysteic acid to yield sulfate under the above conditions is further evidence in favor of the view expressed by Andrews[2] that the formation of sulfate in the oxidation of cystine is through a side oxidation of some intermediate substance preceding cysteic acid. In studies on the oxidation of cystine to cysteic acid in 5.9 N HCl by atmospheric oxygen Andrews found that the oxidation could be greatly accelerated by the addition of Cu^{++} salts whereas Fe^{+++} salts were far less effective. It was noted in this instance that small amounts of inorganic sulfate were formed but that cysteic acid did not yield any. Andrews,[2] furthermore, found that treatment of cysteic acid with pure bromine yielded no inorganic sulfate.

In support of the views expressed by Andrews, Toennies and Lavine[3] found that upon iodine treatment of the partially oxidized cystine obtained by the action of perchloric acid small amounts of sulfate appeared, whereas cysteic acid was entirely stable in the presence of iodine.

In a detailed study of the determination of cysteine and cystine Lugg[4] noted that when either of these compounds was heated with $FeCl_3$ in acid solution that some of the substance disappeared. Lugg states that apparently cysteic acid was formed although it seems no test for cysteic acid was made. From our results it would seem possible that the disappearance of cystine or cysteine as noted by Lugg was at least partly due to the formation of sulfate from the sulfur of these 2 amino acids.

---

2 Andrews, J. C., *J. Biol. Chem.*, 1933, **102**, 263.

3 Toennies, G., and Lavine, T. F., *J. Biol. Chem.*, 1934, **105**, 107.

4 Lugg, J. W. H., *Biochem. J.*, 1933, **27**, 1022.

7668 C

Action of Trichlorethylene on Perfused Vessels of the Frog.

JOHN C. KRANTZ, JR., C. JELLEFF CARR, AND WILLIAM G. HARNE.

From the Department of Pharmacology, School of Medicine, University of Maryland.

Trichlorethylene has had a rather extensive use in the treatment of trigeminal neuralgia since the work of Plessner.[1] Very recently, William Love, Jr., of the Department of Medicine of this university called to our attention the fact that he had successfully treated several cases of angina pectoris by the inhalation of trichlorethylene. Having been interested in the wide use of tissue extracts[2] in the treatment of angio-spastic diseases, the authors thought it would be of interest to study the action of trichlorethylene on the blood vessels and compare it with tissue extract used clinically in the treatment of the same disease.

The experimental procedure developed by Läwen[3] and Trendelenburg[4] for perfusing the vessels of frog's legs was employed. All observations were made during the first 30 minutes of the perfusion. The trichlorethylene was supplied through the courtesy of the Calco Chemical Company and met the requirements set forth by Tschentke.[5] A freshly prepared saturated solution of the substance

TABLE I.

Frog No.	Control Drops per min.	Trichlorethylene 1.5 cc. sat. soln. Drops per min.	Tissue Extract 0.5 cc. Drops per min.	Trichlorethylene 1.5 cc. sat. soln. Drops per min.
1	16	3	14	4
2	21	2	12	3
3	21	4	19	4
4	9	1	6	1
5	27	2	19	11
6	24	5	6	2
7	16	2		
8	4	2		
9	21	9	4	1
10	14	3	4	1
11	16	7	19	4
12	10	2		

1 Plessner, W., *Klin. Wchschr.*, 1916, **53**, 25.

2 Carr, C. J., Schmidt, J. E., Harne, W. G., and Krantz, J. C., Jr., *J. Pharm. and Exp. Therap.*, 1934, **50**, 151

3 Läwen, A., *Arch. Exp. Path. Pharm.*, 1904, **51**, 416. Through Sollmann and Hanzlik.

4 Trendelenburg, P., *Arch. Exp. Path. Pharm.*, 1910, **63**, 165. Through Sollmann and Hanzlik.

5 Tschentke, H. L., *Ind. Eng. Chem. Analyt. Ed.*, 1934, **6**, 21.

in Ringer's solution was employed. The tissue extract was an insulin-free pancreatic extract supplied by Sharp and Dohme, marketed under the name of "Tissue Extract No. 568".

The results of 12 experiments are shown in Table I.

Discussion. Saturated solutions of chloroform in Ringer's solution produced only a slight constricting effect. After the vessels failed to respond any longer to the effect of trichlorethylene, epinephrine solution produced a marked constriction. After tissue extract, pitressin produced no significant constriction of the vessels; but the subsequent administration of trichlorethylene produced marked constriction.

Summary. It is of interest that these therapeutic agents used in the treatment of angio-spastic disease should antagonize the action of each other on the blood vessels of the frog.

7669 C

Reaction of Trichloracetic Acid and of Chloral Hydrate with Carotene.*

VICTOR E. LEVINE AND GEORGE E. BIEN.

From the Department of Biological Chemistry and Nutrition, School of Medicine, Creighton University, Omaha, Nebraska.

Trichloracetic acetic acid (9 parts by weight of the crystallized acid and one part of water) gives immediately with carotene in chloroform solution an intense blue color. The reaction develops without the aid of external heat. Three drops of trichloracetic acid solution are mixed with 0.1 cc. of chloroform solution of carotene in order to develop the reaction. The color fades on the addition of water or of alcohol, but not on heating. Spectroscopic examination reveals absorption beginning at 640mμ and continuing to the end of the visible spectrum.

Carotene in solid form on exposure to air and sunlight changes from copper-colored crystals to a light brown powder. When kept in chloroform solution in a glass bottle exposed to sunlight the deep golden yellow color gradually changes to a light yellow brown. Solid

*The carotene used was obtained from the S.M.A. Corporation, Cleveland. It contains β-carotene and a small quantity of α-carotene. Cerevisterol and ergosterol-free cholesterol were obtained from Dr. Charles E. Bills, Director of Research, Mead Johnson and Company, Evansville, Indiana.

carotene or carotene in solution oxidized by exposure to air and sunlight gives a dirty greenish coloration, turning to dirty grayish on heating. When a sample of carotene in solid form or in solution capable of yielding the above reaction is exposed for a further period, it yields no color reaction with the trichloracetic acid reagent.

Rosenheim[1] reported a specific reaction for ergosterol based upon the procedure we have employed for carotene. Ergosterol gives an immediate red solution showing an absorption band at 500mμ. The red solution gradually changes to a clear blue solution yielding one absorption band at 570-580mμ and another at 650-680mμ. Cholesterol and other sterols give no reaction in the cold. On heating cholesterol in the water bath, however, a red solution develops showing an absorption band at 500 mμ. Rosenheim was able to detect by the use of trichloracetic acid at room temperature the presence of ergosterol in cholesterol. McDonald and Bills[2] demonstrated a series of color changes for iso-ergosterol with the Rosenheim reagent. Honeywell and Bills[3] reported that cerevisterol reacts with the Rosenheim reagent yielding with minute amounts of this sterol an initial red color, which soon fades, and with higher amounts a red color which gives way to a dirty green or even black coloration. With the Rosenheim reagent we observed with cerevisterol a pink coloration changing to dark brown. When the test with cerevisterol is made by heating the reaction mixture on a water bath, an evanescent light red color is formed, giving way to brown and finally to dark purple.

It is evident that the Rosenheim procedure for testing ergosterol applies equally well to carotene. The carotenoid pigment, however, is more sensitive to the trichloracetic acid reagent. Rosenheim reported the limit of sensitivity for ergosterol to be 0.005 mg., using 3 drops of trichloracetic acid and 0.1 cc. chloroform solution of the sterol. Under the same quantities of reagent and chloroform solution we found the limit of sensitivity to be 0.001 mg. for ergosterol and 0.0002 mg. for carotene. The tests given by the 2 compounds show marked differences. With ergosterol an initial red color is obtained and a final blue color; with carotene no preliminary red color appears and the blue color develops immediately. Examination of the reaction mixtures with the spectroscope reveals differences in the absorption bands in the visible spectrum.

Chloral hydrate also reacts with carotene. About 0.5 gm. of chloral hydrate is liquefied by placing in a small evaporating dish

[1] Rosenheim, O., *Biochem. J.*, 1929, **23**, 47.

[2] McDonald, F. G., and Bills, C. E., *J. Biol. Chem.*, 1930, **88**, 601.

[3] Honeywell, E. M., and Bills, C. E., *J. Biol. Chem.*, 1932, **99**, 71.

and heating on the water bath. One drop of concentrated hydrochloric acid and finally 0.1 cc. of a solution of carotene in chloroform are added. An intense blue color forms immediately. The color fades on the addition of water or alcohol. The limit of sensitivity following the above procedure is 0.001 mg. of carotene in 0.1 cc. of chloroform. The concentration of acid in the reaction mixture modifies the test. A chloroform solution of carotene mixed with an equal volume of concentrated hydrochloric acid yields at first a yellow brown color, changing to olive green and finally to light green. Solid carotene dissolves in liquefied chloral hydrate with the formation of a blue solution.

Rosenheim applied chloral hydrate as a reagent for ergosterol. He reported in 1929 that when solid ergosterol (1 mg. or less) is added to liquefied chloral hydrate (0.5 gm.), there develops a carmine red solution, changing within a minute to an evanescent green and finally to a deep blue, which persists for a considerable time. The test he reported to be specific for ergosterol since carefully

ERRATUM

Page 337 "sisterol" should read "sitosterol".

reagent. We have found, however, that ergosterol-free cholesterol gave when heated with chloral hydrate on the water bath a pink or reddish fluid.

An acid reaction, according to Rosenheim, is essential to the reaction. A saturated solution of chloral hydrate (80%) in water yielded a positive test with ergosterol when a drop of concentrated hydrochloric acid was added. Freshly distilled anhydrous chloral gave a positive test with ergosterol. The addition of a drop of water activated the mixture. Chloral or chloroform under laboratory conditions may undergo slight decomposition with the formation of traces of hydrochloric acid.

Conclusion. Trichloracetic acid and chloral hydrate are reagents that serve in the detection of ergosterol and of carotene. Ergosterol with the 2 reagents yields an initial red and a final blue color, while carotene gives only the blue color.

7670 P

Increase in Lymphocytes in Healthy Persons Under Certain Emotional States.

EDMOND J. FARRIS. (Introduced by M. X. Sullivan.)

From the Department of Anatomy, Georgetown University School of Medicine, and the Department of Anatomy, Medical College of the State of South Carolina.

The purpose of this preliminary report is to call attention to a relative lymphocytosis observed in healthy individuals under certain emotional states. Menkin[1] showed that excitement in cats of 10 to 15 minutes duration caused a relative emotional increase in mononuclears, averaging 13% with a return to normal within 30 minutes after. As far as known, no other similar investigations have been reported.

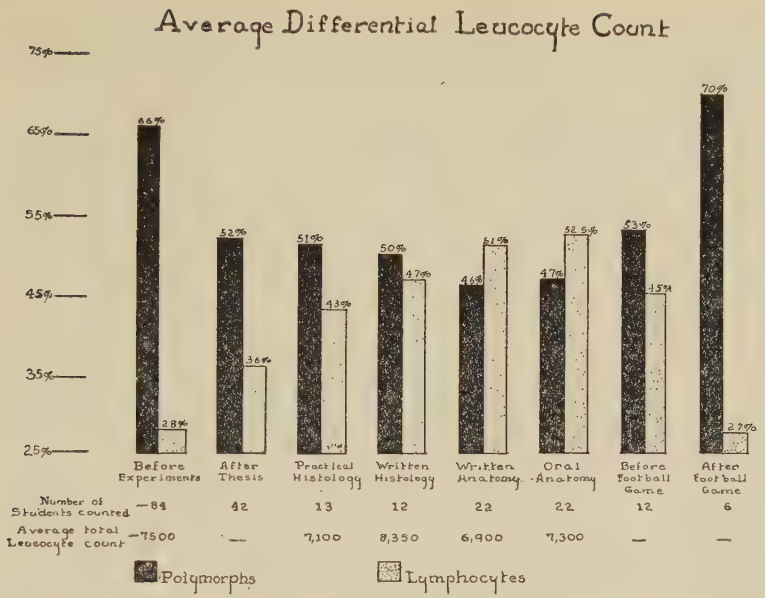
Freshman medical students and members of a football team, all apparently in good health, were used. All blood samples were drawn from the fingers, and the smears stained with Wright stain. Two hundred cells per smear were counted and the percentage of lymphocytes determined. In the preliminary work, total leucocyte counts were made in every case, but not continued because no departure from the normal count was in evidence. The experiments were carried on for 3 successive years with respect to medical students, and one season only with respect to the football team.

Blood counts were made on the medical students at the beginning of each session, and a normal for each class established. During the final examinations, blood counts were made as follows: (1) a group during the practical histology examination, (2) a group during the written anatomy examination, and (3) a group during the oral anatomy examination. Also, a differential leucocyte count was made on the members of one of these classes on the completion of an assigned thesis. On the members of the football team, blood counts were made approximately one-half hour preceding the start of a match game, and approximately one-half hour after the end of the same game.

The results of the experiments showed percentage increases in lymphocytes as indicated in the chart. Examination of this chart, columns (3-6), indicates clearly that as the emotional strain was continued, the lymphocyte count rose correspondingly.

The physiology of the relative lymphocytosis cannot be explained at present. The work of E. F. Muller[2] and associates, and of

[1] Menkin, V., *Am. J. Physiol.*, 1928, **85**, 489.



Camp[3] and others suggests a complex reaction of the nervous system.

Summary. Emotional states produce a marked relative lymphocytosis in healthy persons.

7671 P

Note on Absorption of Phage by Heat-Killed Bacilli.

PHILIP LEVINE AND ARTHUR W. FRISCH.

From the Department of Pathology and Bacteriology, University of Wisconsin, Madison.

Investigations on the Salmonella were carried out employing the method recently described by us[1] for studying the specificity of absorption, particularly from polyvalent phages, by heat-killed bacilli. In principle the method depends on the fact that the absorption of phage by heat-killed bacilli is largely irreversible and consequently the residual phage may be studied both qualitatively and quantitatively without removing the absorbing organisms.

---

[2] Muller, E. F., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 95.

[3] Camp, W. J. R., *J. Lab. and Clin. Med.*, 1927, **13**, 206.

[1] Levine, Ph., Frisch, A. W., and Cohen, E. V., *J. Immunol.*, 1934, **26**, 321.

TABLE I.

Tests with anti-*B. Paratyphosus A* phage. Test Organism—*B. aertrycke.*

	Dilutions of Phage					
Absorbed with heat-killed	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
B. aertrycke	+++±	++++	++++	++++	++++	++++
B. Suipestifer (European)	+±	+++±	++++	++++	++++	++++
B. Suipestifer (American)	Cl	Cl	Cl	Cl	+++	++++
Saline	Cl	Cl	Cl	Cl	Cl.	++++

The turbidity reading recorded was made 6½ hours following the addition of the test organism.

Cl. indicates complete clearing; ±, +, +±, ++, etc., indicate increasing degrees of turbidity.

In general, it was possible with this procedure to differentiate some of the following groups, for instance, the suipestifer group which absorbed specifically the homologous phage and also the suipestifer fraction[1] of the polyvalent anti-paratyphosus A phage. However, some of the suipestifer strains, particularly those of the European type, were characterized by a capacity to absorb actively also other fractions, for example, the paratyphosus B fraction of the polyvalent anti-paratyphosus A phage when *B. aertrycke* (of the paratyphosus B group) was used to test for residual phage. (Table I.) This same type of suipestifer absorbed the anti-paratyphosus B phage to a moderate degree, but less intensely than the paratyphosus B group of organisms, while other suipestifer strains showed no absorption.*

The paratyphosus A group of organisms and also some of the European suipestifers employed were the most active absorbers of the anti-paratyphosus A phage when the homologous strain was the test organism. The distinction between these could be made readily since the paratyphosus A strains failed to absorb actively the anti-suipestifer phage.

In contrast to the ease with which differences within the suipestifers could be demonstrated, was the failure to distinguish the typhosus-enteritidis group from the aertrycke-paratyphosus B group, since in the several combinations of phages and test organisms available, both groups absorbed equally well. (Presumably this observation is in harmony with the findings recorded by Furth and Landsteiner,[2] that some anti-sera of the 2 groups gave weak to moderate precipitin reactions also with the heterologous extract.) Nevertheless, extracts of the 2 groups could be differentiated by

* From other experiments evidence was obtained to indicate the existence of still further differences in the suipestifer group, apparently not connected with the biochemical types.

[2] Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1929, **49**, 727.

phage, since greater specific inhibition of the anti-paratyphosus B phage was obtained depending upon the group of the test organism employed.[3]

A discussion of the significance of the observations made and their bearing on the nature of bacteriophage specificity is reserved for future publication.

7672 C

Observations on Phage Inhibition by Bacillary Extracts.

PHILIP LEVINE AND ARTHUR W. FRISCH.

From the Laboratories of the Department of Pathology and Bacteriology, University of Wisconsin, Madison.

The specific inhibition of bacteriophage by bacillary extracts recently described by us[1, 2] and confirmed by Kligler and Olitski,[3] Burnet,[4] and Gough and Burnet[5] may be employed to study the chemical properties of these complex cellular products with the aid of various manipulations such as fractionation and hydrolysis.

In a previous communication,[2] the observation was made that crude saline extracts of *B. aertrycke* boiled in N/2 alkali, still retained their phage inhibiting capacity and, consequently, it seemed desirable to study in detail the sensitivity of these substances to alkaline and acid treatment before attempting purification. For this purpose, a crude saline extract of *B. aertrycke* was employed and tested for phage inhibition before and after boiling in N/2 sodium hydroxide for 5 minutes* and subsequent neutralization. Such alkali treated extracts showed marked increase in phage inhibiting capacity although they gave a precipitin titre that was slightly lower than that of the untreated material. This observation, therefore, indicated that the degree of inhibition of phage did not run parallel to the precipitin titre of the crude extract. Striking confirmation of

[3] Levine, Ph., and Frisch, A. W., *J. Exp. Med.*, 1934, **59**, 213.

[1] Levine, Ph., and Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 993; **31**, 46.

[2] Levine, Ph., and Frisch, A. W., *J. Exp. Med.*, 1934, **59**, 213.

[3] Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1934, **59**, 213.

[4] Burnet, F. M., *J. Path. and Bact.*, 1934, **38**, 285.

[5] Gough, G. A. C., and Burnet, F. M., *J. Path. and Bact.*, 1934, **38**, 301.

* Under such conditions an inactive precipitate appeared which was removed after neutralization.

this view was found when these extracts were subjected to mild hydrolysis in N/15 and N/30 hydrochloric acid at 80°C. This procedure was adopted because boiling for 5 minutes or longer in N/2 acid completely destroyed both the phage inhibiting reaction and also the capacity to react with precipitins.

A crude extract of *B. aertrycke* was dissolved in saline, passed through a Seitz filter and then treated with N/5 hydrochloric acid in sufficient quantity to produce a final concentration of N/15 acid and held at 80°C. Samples were removed at various intervals, neutralized, brought to a dilution of 1:500, centrifuged to remove the precipitates, and sterilized by heating for one hour at 80°C. These solutions were then tested for their capacity to inhibit the action of the anti-paratyphosus B phage by means of the following method, a modification of the original adopted in order to permit the use of small quantities of extract. One drop of a 1:500 sterile solution of the extract was mixed with one drop of varying dilutions of the anti-paratyphosus B phage in serological tubes (100 mm. long and 20 mm. in diam.) and allowed to incubate over night at 37°C. (wet). The next day 2 cc. of beef extract broth was added to the sterile mixtures followed by one drop of a young broth suspension of *B. paratyphosus B*. Frequent turbidity readings were made to study the course of the lysis.

The results showed that specimens removed after treatment with mild acid for 30 to 90 minutes and somewhat longer intervals gave striking inhibition effects, considerably stronger than that of the untreated material. These solutions, however, showed a progressive diminution in their reactivity with precipitins (Table I). Somewhat

TABLE I.

Aertrycke extract	Precipitin titer with anti-aertrycke rabbit immune serum: Dilutions of extract 5,000	50,000	200,000	Intensity of phage inhibition
Untreated	++++	+++	++	+±
30 min. in N/15 acid	+	+	f. tr.	++±
90 min. in N/15 acid	+	f. tr.	0	++++
4 hr. in N/15 acid	±	0	0	+±

analogous results were obtained with material previously boiled in N/2 alkali and subsequently subjected to similar acid treatment.

The data suggest the hypothesis that mild treatment with acid results in the appearance of partial hydrolytic products in which the specific reacting groups are unmasked as disclosed by the more intense phage reaction. This explanation seems likely, especially in

TABLE II.

Aertrycke extract	Precipitin titer. Dilutions of extract 5,000	50,000	500,000	5,000,000	Tested with antibody derived from:
Untreated	+++	++±	+±	±	Rabbit serum
	±*	+	+	tr	Horse "
90 min. in N/15 acid	±	tr	0	0	Rabbit "
	+±	+±	±	0	Horse "

*Zone effect.

Precipitin reactions recorded were made after the tests stood 2 hours at room temperature and over night in the ice box.

view of the characteristic behavior of the acid-treated solutions when tested with antibodies derived from rabbit and horse, namely better precipitin reactions with the anti-*B. paratyphosus B* horse serum (Table II). The assumption that the observations made are due to the appearance of hydrolytic products is further suggested by a similar finding of Heidelberger and Kendall,[6] made with isolated hydrolytic products of the specific soluble substance of Pneumococcus type III, which gave precipitin reactions with specific antibodies obtained only from the horse and not from the rabbit.

The lack of parallelism between the precipitin reaction and phage inhibition may be understood if one assumes that for a precipitin reaction, as a rule, substances of higher molecular weight are required; whereas, specific inhibition reactions in general may be brought about by simpler chemical substances. (Landsteiner.)

Conclusive tests to determine whether the phage inactivating solutions obtained by mild acid treatment inhibit the precipitin reaction with rabbit antibodies, must await studies on the isolation and purification of the active phage inhibiting substances.

7673 C

Inactivation and Regeneration of the Glycolytic Enzyme System of Muscle Extract.

L. MICHAELIS AND J. RUNNSTRÖM.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

An aqueous extract of muscle tissue, prepared according to Meyerhof,[1] contains the glycolytic enzyme which produces lactic acid

[6] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

[1] Meyerhof, O., *Biochem. Z.*, 1926, **178**, 395.

from carbohydrates. This enzyme is very labile and loses its activity quickly on standing. Recently Lipmann[2] advanced the idea that this enzyme is easily oxidized, is inactive in its oxidized form, and can be reactivated by reduction. This idea led him to a theory of what has been called the Pasteur reaction. The fact that a cell produces lactic acid as end product when utilizing carbohydrate anaerobically, but much less or no lactic acid under aerobic conditions, has led Meyerhof to assume that lactic acid is also produced aerobically, but resynthesized to glycogen, the energy of the synthesis being furnished by the respiration process. Lipmann, on the other hand, assumes that lactic acid is not produced aerobically, or to a smaller extent, owing to the fact that aerobically the lactic acid enzyme is present in its oxidized inactive state. Though Lipmann's theory is still open to question, one part seems certain, namely that the lactic acid enzyme is easily oxidized and thereby loses its activity. Holmberg[3] tries to explain Lipmann's results on the following basis: When an oxidant is added to the system the enzyme is not oxidized, but the presence of the oxidant prevents the reduction processes necessary for the formation of lactic acid (formation of glycerol phosphoric acid and reduction of pyruvic acid). Thus only the oxidation products such as phospho-glyceric acid can be formed. This interpretation, however, cannot be accepted, because the formation of this acid also would be recorded by the manometric method used by Lipmann.

There is also evidence from Lipmann's experiments that the inactive enzyme might be reactivated by reduction, but this evidence is, as yet, not very strong. He succeeded in restoring the previously oxidized enzyme to a certain extent by ascorbic acid, but the effect is poor. In his experiments the reactivation does not raise the effect of the enzyme to its original value. This original value, however, is very likely smaller than the maximum value that would be obtained if all the enzyme were in the active form. A regenerating agent should at least restore the original activity of the fresh muscle extract and even raise it above this level, since, even in a fresh extract a part of the enzyme might have already undergone inactivation.

This paper shows that the glycolytic enzyme of muscle extract, even after it has become entirely inactive by standing for several days, can be reactivated by thioglycolic acid to an extent exceeding the activity in the freshly prepared extract.

---

[2] Lipmann, F., *Biochem. Z.*, 1933, **265**, 133.

[3] Holmberg, C. G., *Skand. Arch. f. Physiol.*, 1934, **68**, 1.

Other sulphydril compounds could have been used, but thioglycolic acid has the great advantage of being rather stable when exposed to the air. The whole system, *viz.*, muscle extract + coenzyme + glycogen + buffer + reductant must be prepared in the microrespiration vessel exposed to the air. The pH of the system being 7 to 8, the conditions for autoxidation of sulphydril compounds such as cysteine are most favorable, and it is difficult to prevent the added reductant from being oxidized before the air in the chamber has been displaced by the N_2-CO_2 mixture. Thioglycolic acid is much more slowly oxidized under these conditions even in presence of a considerable amount of iron, and is therefore preferable as a reductant.

The problem arises as to the nature of the enzyme or at least of the oxidizable and reducible group of the enzyme. Among those substances which inactivate the enzyme is iodoacetic acid. This acid has been shown by Lundsgaard[4] to suppress the formation of lactic acid even in the living animal, and Lipmann finds it inactivates the enzyme contained in the muscle extract. Lipmann ascribes this property of iodoacetic acid to its oxidizing faculty. He simply enumerates this acid as one among the oxidants used. However, iodoacetic acid is no oxidant at all. Von Euler[5] advances the hypothesis that iodoacetic acid might act due to its reaction with copper. Such an interaction, however, can not be demonstrated experimentally. On the other hand, iodoacetic acid exhibits an almost specific reaction to sulphydril compounds.[6] It exhibits another reaction towards amino groups.[7] These reactions consist in substituting the radical CH_2 COOH for H in the SH or in the NH_2 group. In general the reaction with SH groups is faster and goes on in less alkaline solutions than the reaction with NH_2 groups. A quick reaction in an approximately neutral solution may be taken as a fairly strong evidence that a SH group is concerned and may be taken as a full proof if corroborated by other evidence. We have the following facts pertaining to this problem: (1) The enzyme is, according to Lipmann, inactivated by oxidation (quinone, oxygen, suitable dye stuffs). (2) The enzyme is also inactivated by iodoacetic acid. (3) The enzyme inactivated by exposure to air is regenerated by thioglycolic acid.

---

[4] Lundsgaard, E., *Biochem. Z.*, 1933, **27**, 1141.

[5] von Euler, H., *Ergebnisse der Enzyrnforschung* 111, Leipzig, 1934, edited by F. Nord and Weidenhagen.

[6] Dickens, F., *Biochem. J.*, 1933, **27**, 1141.

[7] Michaelis, L., and Schubert, M. P., *J. Biol. Chem.*, 1934, **106**, 331.

These facts appear to be fair evidence for the hypothesis that the enzyme is a sulphydril compound, active as an enzyme only in its sulphydril form, but inactive in its disulphide form.

This is another case where the activity of an enzyme is correlated with a sulphydril group which is obviously a constituent of the enzyme itself, and where the oxidation of this group is correlated with the inactivation of the enzyme.

Various enzymes have been known which can be activated by potassium cyanide or hydrogen sulphide [Papain, Kathepsin, Arginase, Urease (Grassmann[8] and Waldschmidt-Leitz[9]).] The mode of action of these activators was sometimes interpreted by the assumption that these substances were to eliminate traces of heavy metal salts which exhibit an inhibitory influence (Krebs[10]). Another interpretation was the assumption that glutathione, in its reduced form has the function of a coenzyme, and that oxidized glutathione is reduced by these agents (Perlzweig[11]). What seems to us the first appropriate interpretation of the nature of the sulphydril group concerned is the one given by Sumner and Poland,[12] who showed that the sulphydril group concerned with the action of urease is not that of glutathione but that the enzyme itself contains a sulphydril group. This idea has been more fully and very convincingly developed by Hellerman, Perkins, and Clark,[13] who gave evidence for the hypothesis that the activity of the enzyme is correlated with a sulphydril group of the enzyme molecule itself. At the same time Bersin and Logemann[14] developed the same idea for Papain. The case of the glycolytic enzyme, as here presented, can be added to that group of enzymes which contain a sulphydril group and are active only when this group is present in its reduced state.

The extract was prepared from guinea pig muscles which immediately after the dissection were put on ice. The muscles were cut up in small pieces and extracted with 1.5 times their weight of water, the extract filtered and some drops of octyl alcohol added to keep it approximately sterile for several days. In some experiments fresh mutton meat was used, with similar results.

---

[8] Grassmann, W., Schoenbeck, O., and Eibeler, H., *Z. Physiol. Chem.*, 1930, **194**, 124.

[9] Waldschmidt-Leitz, E., and Purr, A., *Z. Physiol. Chem.*, 1931, **198**, 260.

[10] Krebs, H. A., *Biochem. Z.*, 1930, **220**, 289.

[11] Perlzweig, W. A., *Science*, 1932, **76**, 435.

[12] Sumner, J. B., and Poland, L. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 553.

[13] Hellerman, L. H., Perkins, M. E., and Clark, W. M., *Proc. Nat. Acad. Sc.*, Washington, D. C., 1933, **19**, 855.

[14] Bersin, T., and Logemann, W., *Z. Physiol. Chem.*, 1933, **220**, 209.

The coenzyme used was prepared according to Lohmann.[15] We did not, however, attempt to purify it but used the crude preparation which turned out to have a high content of adenylpyrophosphate and to be very active.

The glycolysis was measured in the Warburg apparatus. 10 cc. of the muscle extract were mixed with 1 cc. of 0.85% $NaHCO_3$ solution, and 2 cc. of this mixture used for each Warburg vessel. In addition 0.2 cc. coenzyme and 0.1 to 0.3 cc. of an approximately molar neutralized solution of thioglycolic acid, or, for the control experiment, an equivalent amount of water, was added. The side arm of the vessel was filled with 0.2 cc. of a 0.2% solution of starch, or, in most experiments, glycogen. In some experiments, a 0.18 molar solution of hexosediphosphate was used as a substrate. The gas space of the apparatus was filled with a mixture of purified nitrogen with 5%, or in most experiments, 10% CO_2 (causing a pH of about 7.8 in the solution). After the first reading the substrate was tipped into the main compartment.

As the manometer readings were used only comparatively, no phosphorylation correction (Meyerhof) was applied. Only a "retention factor", R, amounting to 1.4 has been applied in the tables besides the immediate readings. The experiments recorded here are a few among a great number carried out.

The chemical determination of lactic acid was carried out essentially by the method of Friedemann, Cotonio and Shaffer, with the modification recommended by Wendel.[16] It may be mentioned that in presence of thioglycolic acid, special care should be taken that a sufficient amount of copper sulphate is used at that stage of the procedure, where the deproteinized filtrate is mixed with calcium hydroxide + $CuSO_4$ to eliminate those substances which might interfere with a sharp endpoint of the final iodine titration of the distillate.

A freshly prepared muscle extract is usually active. A mixture of such an extract + glycogen, without coenzyme or still better with coenzyme added, produces acid. On addition of thioglycolic acid this effect is distinctly increased. (Table I.)

TABLE I.

Muscle extract (cc.)	2	2	2	2
Coenzyme (cc.)	0	0.2	0	0.2
Thioglycolic acid (cc.)	0	0	0.3	0.3
Glycogen (cc.)	0.2	0.2	0.2	0.2
CO_2 (cbmm. in 40 min.)	119	126	272	297
R × CO_2	167	176	380	415

[15] Lohmann, K., *Biochem. Z.*, 1931, **233**, 460.

[16] Wendel, W. B., *J. Biol. Chem.*, 1933, **102**, 47.

The activity of this muscle extract gradually decreases and drops to zero within the first day. On the second or third day, there was never any activity with glycogen as substrate, but the activity could be entirely regenerated by thioglycolic acid (Table II). The activity towards hexose phosphate persisted to a certain extent to the second day but was always increased by addition of thioglycolate (Table III).

Finally it was shown that the acid formed was essentially lactic acid (Table IV).

The amount of lactic acid actually produced is smaller than the acid produced according to calculation from the manometric results. This may be accounted for by the fact that the phosphorylation which causes a shift to the acid side (Meyerhof[1]) is faster than the formation of lactic acid. This is shown by Table V.

TABLE II.

Muscle extract (cc.)	2	2	2	2
Coenzyme (cc.)	0	0.2	0	0.2
Thioglycolic acid (cc.)	0	0	0.3	0.3
Glycogen (cc.)	0.2	0.2	0.2	0.2
CO_2 (cbmm. in 60 min.)	2	17	159	204
R $\times$ CO_2	3	24	223	286

TABLE III.

Muscle extract (cc.)	2	2	2	2
Coenzyme (cc.)	0	0.2	0	0.2
Thioglycolic acid	0	0	0.2	0.2
Glycogen (cc.)	0.2	0.2	0	0
Hexosediphosphate (cc.)	0	0	0.2	0.2
CO_2 (cbmm. in 60 min.)	0	4	62.5	129.5
R $\times$ CO_2	0	6	87.5	182

TABLE IV.

Muscle extract (cc.)	10	10	10	10
Glycogen (cc.)	1	1	1	1
Coenzyme (cc.)	1	1	1	1
Thioglycolic acid (cc.)	0	0	1	1
Duration of exp. (min.)	0	105	0	105
Lactic acid (mg.)	3.5	4.2	3.4	5.2
Increase of lactic acid, %		20		53

TABLE V.

Muscle extract (cc.)	2	2	2	2
Glycogen (cc.)	0.2	0.2	0	0
Hexosediphosphate (cc.)	0	0	0.2	0.2
Coenzyme (cc.)	0.2	0.2	0.2	0.2
Thioglycolic acid (cc.)	0.3	0.3	0.3	0.3
Duration of exp. (min.)	0	120	0	120
Mg. inorganic P/1cc. extract	0.257	0.068	0.247	0.318

In the course of the experiment with glycogen as substrate almost 75% of the inorganic phosphate initially present has been esterified. Table V shows also that the content of inorganic phosphate increases, when hexose phosphate is used as substrate, in accordance with expectation.

Summary. The glycolytic enzyme of muscle extract after becoming inactive on standing can be reactivated by thioglycolic acid to a higher state of activity than that originally present. Lipmann's hypothesis of the enzyme being inactivated by oxidation and reactivated by reduction is corroborated and specified by the evidence that this oxidation-reduction is concerned with a sulphydril group of the enzyme. There is evidence for the assumption that glycolytic enzyme of muscle seems to be a sulphydril compound which loses its enzymatic property by oxidation to the disulphide state and is reactivated by reduction to the sulphydril state.

7674 P

Pharmacological Studies on the Blood of Trachoma.

DAVID I. MACHT.

From the Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

The writer has reported his phytopharmacological studies on blood sera from various clinical conditions and described characteristic phytotoxic reactions produced by such specimens from certain diseases. These reactions were found useful in establishing a differential diagnosis and also in evaluating the results of therapeutic procedures.[1] Such specific phytotoxic reactions were particularly noted in pernicious anemia,[2] leprosy,[3] and pemphigus.[4, 5] This method of investigation has been repeatedly employed by Professor Leon Tscherkes of Odessa, who has confirmed Macht's findings. Some time ago, in a personal communication to the author, Professor Tscherkes wrote that he had discovered that blood sera from still another pathological condition exhibited a marked phytotoxic property for the seedlings of *Lupinus albus*—namely, trachoma. Since

1 Macht, *Science*, 1930, **71**, 302.

2 Macht and Anderson, *J. Pharmacol. and Exp. Therap.*, 1928, **34**, 365.

3 Macht, *Acta Dermat.*, 1932, **18**, 126.

4 Pels and Macht, *Arch. Dermat. and Syph.*, 1929, **19**, 640.

5 Pels and Macht, *Arch. Dermat. and Syph.*, 1931, **23**, 601.

this discovery was of considerable importance, the present writer undertook a study of blood sera from a series of trachoma cases in the United States in order to corroborate the findings of Tscherkes.

Specimens of blood from trachoma patients in all stages of the disease were obtained from different sources. A few blood sera were obtained from sporadic cases in Baltimore; for the most part, however, specimens were secured through the courtesy of public health officers in Tennessee, Kentucky and Indian Territory. According to the method described in detail elsewhere,[6] these blood sera were tested on seedlings of *Lupinus albus;* and the phytotoxic indices of trachoma blood were compared with those given by normal human sera and by sera from various other ophthalmological diseases obtained, with permission of Professor Wilmer, from the Wilmer Institute of the Johns Hopkins University. The results obtained with specimens from 100 trachoma cases are indicated below. At the time of examination, in 30 cases, the disease had endured for periods ranging from one month to one year; in 10 cases, it had persisted from 10 to 20 years; in 8 cases for more than 20 years; and, in 3 cases, for more than 40 years. The course of the disease in the remainder of the 100 patients suffering with trachoma varied from 1 to 10 years. The average phytotoxic index of the trachoma sera studied was 47.7%, as compared with an index of 72% for normal human blood. The range of toxicity in various cases was as follows: Specimens from 2 cases gave a reading of 22%; and blood serum from another case, a phytotoxic index of 28%. The high toxicity of these sera was paralleled only by that of specimens from virulent cases of leprosy. Specimens from 17 patients exhibited phytotoxic indices ranging from 30 to 40%; and sera from 41 cases gave readings varying from 40 to 50%. This range of toxicity was comparable to that exhibited by specimens from pernicious anemia cases. The sera from 28 trachoma cases gave readings varying from 50 to 60%, exhibiting almost the same range of toxicity as is shown by specimens from the average run of pemphigus patients. Sera from 8 cases gave readings ranging from 60 to 70%; and specimens from only 3 cases exhibited indices of 70% or more—in other words, showed no toxic reaction. There was apparently no relation between the toxicity of blood specimens and the duration of the disease. Specimens from acute cases, however, seemed to exhibit a greater toxicity than sera from chronic patients. Control experiments were repeatedly made both

[6] Macht and Livingston, *J. Gen. Physiol.*, 1922, **4**, 573.

with normal blood sera and with sera from various other pathological eye conditions, particularly acute and chronic conjunctivitides. These uniformly gave readings of from 70 to 75%, or the same phytotoxic index as normal blood. Specimens of blood from three cases of glaucoma also gave normal readings of 75%.

From the standpoint of etiology and pathology of trachoma, these findings are of considerable interest, especially as the causation of that disease is still in dispute. They tend to support the view that the disease is not a simple local affection of the eyelids but is associated with profound systemic derangement in metabolism, which produces a sort of toxemia, at least as far as the toxicity of the blood serum for living plant protoplasm is concerned. Here it may be well to observe that Macht and Pels have examined the blood sera from a number of cases of pemphigus of the eye, which also gave a phytotoxic reaction. On the basis of the blood examination alone, differential diagnosis between pemphigus of the eye and trachoma is rather difficult to establish. However, pemphigus can be easily distinguished from trachoma if, on further examination, lesions are revealed on other parts of the body. A more detailed account of the present investigation will appear in the *Folia Ophthalmologica Orientalia.*

7675 P

Parasympathetic Drugs and Ovulation.*

MARK A. FOSTER, HANCE F. HANEY AND F. L. HISAW.

From the Departments of Zoology and Physiology, University of Wisconsin.

Studies on the nervous mechanism involved in copulatory ovulation have disclosed several interesting facts. First, the sympathetic chain has been shown not to be essential for ovulation and pregnancy in the cat[1] or rat.[2] Secondly, mid-brain removal or section of the brain stem immediately after copulation does not prevent ovulation in the rabbit if the anterior pituitary is left intact.[3] These facts suggest that a nervous stimulus reaches the pituitary during copulation and that the pathway is probably independent of the sympathetics.

* Aided in part by a grant from the National Research Council, Committee on Problems of Sex.

1 Cannon, W. B., *et al., Am. J. Physiol.*, 1929, **89**, 84.

2 Bacq, Z. M., *Am. J. Physiol.*, 1932, **99**, 444.

3 Fee, A. R., and Parkes, A. S., *J. Physiol.*, 1927, **67**, 383.

The purpose of this study has been to determine the effect of parasympathetic drugs on the process of ovulation. The first step was to determine whether or not these drugs alone would cause ovulation.

Adult female rabbits having been isolated 2 to 5 weeks and showing the typical vulval swelling and purple congestion of full oestrus were used. Four were treated with atropine sulfate (2½ mg./kg. intravenously); 2 with pilocarpine (1-3 mg./kg. every 2 hours for 6 hours); and 3 with physostigmine (¼ mg./kg. every hour for 6 hours). Laparotomy was performed in each case 48 hours after the last injection. Ovulation was not induced in any case. Ovaries in many animals showed signs of a decrease in oestrin secretion as was indicated by paling of the uteri and vulva. These rabbits, instead of ovulating, went out of oestrus immediately and refused to copulate for several days. This indicates that these drugs are inhibitory in their action rather than stimulatory.

TABLE I.
Atropine Series.

Animal	Dosage	Time of copulation after injection	Results
582	2½ mg /Kg.	10 to 30 sec.	3 to 4 ovulation† points in each ovary
A1	1.7 mg./Kg.	3 min.	4 to 5 points on each ovary†
574	2½ mg./Kg.	3½ "	no points, hemorrhagic cysts
584	"	4 "	4 points in both ovaries†
581	"	5 "	No points, ovary normal
579	"	6 "	
579B	"	9 "	
580	"	9 "	
A2	"	10 "	
583	1¼ mg./Kg.	15 "	
580B	2½ mg./Kg.	20 "	

†These females ovulated after copulation, but never bore young.

To test this hypothesis, a series of 11 rabbits was injected with atropine as in the former series and allowed to copulate. The time which elapsed before copulation was different for each individual (1, 3, 4, 5, 7, 10 minutes, etc.), so that a complete series was obtained in which the time of drug action varied from 1-30 minutes. From the results shown in Table I it is evident that ovulation occurs normally if the drug acts for less than 4 to 5 minutes. Longer action completely inhibited ovulation in all cases. This suggests 2 possibilities for the mechanism of inhibition. Either the drug is acting directly on the graafian follicle or on the pituitary. To test the former possibility minimal and subminimal ovulating dosages of purified pituitary extracts were injected into atropine-treated ani-

mals. In all cases the ovulation reactions were normal, thus showing the follicles were capable of ovulating. However, the M.O.D. for atropine-treated animals was higher than normals.

A group of 6 rabbits was treated with pilocarpine (3 mg./kg. and 2 mg./kg.). Ovulation was inhibited with the larger dosage in 3 cases out of 4 after it had acted 18 minutes or longer, but since this dosage approaches the M.L.D. these results may be questioned. No inhibition was obtained in 2 cases on a lower dosage of 2 mg./kg. Physostigmine acts similarly to pilocarpine.

It is of interest to note that even though ovulation occurred in 6 rabbits which copulated within 1-4 minutes after atropine injection no young were born. The same was true of one case treated with 3 mg./kg. of pilocarpine in which ovulation occurred.

7676

Antuitrin-S Effect upon Blood Elements.*

SAMUEL H. GEIST AND FRANK SPIELMAN.

From the Gynecological Service, Mount Sinai Hospital, New York City.

Novak[1] has stated that the good results he has obtained from the administration of the anterior pituitary-like hormone (Antuitrin-S) in cases of functional uterine bleeding were possibly due to changes produced in the blood by the action of this substance. To determine the presence or absence of such changes, 6 patients were given Antuitrin-S by hypodermic injection, using 1 cc. 3 times weekly. Three of these were injected for a period of 2 weeks, the other 3 for 3 weeks. Complete blood examinations (involving the formed elements only) were made before the injection of the product was begun, and again after all the injections had been completed. These examinations included the hemoglobin, red blood count, white blood count and differential, platelet count, bleeding time, coagulation time, and tourniquet test. It was felt that comparison of the figures obtained before and after treatment might throw some light upon the effect of Antuitrin-S upon the blood elements, as well as explain any improvement in functional uterine bleeding noted, if dependent on blood changes. The patients selected were all fertile women who

* The authors wish to express their gratitude to Dr. Nathan Rosenthal who carried out the blood examinations.

1 Novak, E., Hard, G. B., *Am. Gynecological Soc.*, 1931, **56**, 146.

presented no demonstrable abnormalities other than the uterine bleeding.

The study of the results of the injections demonstrated that the effect on the blood was so slight as to be negligible. In most cases there was some drop in the hemoglobin after the administration of the hormone, the greatest difference being 13% and in only 1 case a rise of 4%. The other variations were too unimportant to require comment. It is interesting to note that the platelet count almost uniformly was lower after treatment. This is surprising, if the fact that a reduction in platelets is usually found associated with an increased tendency toward hemorrhage, is accepted. As has been reported elsewhere, we have not been able to obtain cessation of bleeding in cases of functional metorrhagia with large doses of Antuitrin-S. It is needless to add that the patients studied in the above series showed no improvement from the standpoint of the bleeding. The continued bleeding may even explain the uniformity in diminution of the hemoglobin.

From the above studies we concluded the injections of Antuitrin-S did not affect the formed blood elements.

7677 C

Basic Amino Acids Yielded by Thyroglobulin.*

ABRAHAM WHITE AND WILLIAM G. GORDON.†

From the Department of Physiological Chemistry, Yale University, New Haven.

The present analysis of thyroglobulin was undertaken in order to obtain information by modern isolation methods regarding its basic amino acid composition. The preparation‡ was made by the method of Barnes[1]; the low phosphorus content (Table I) indicates that little impurity of a nucleoprotein nature could have been present. The iodine content is slightly higher than that reported by Heidelberger and Palmer[2] but is considerably lower than Oswald[3]

* Grateful acknowledgement is made to Dr. H. B. Vickery for aid in the preparation of this manuscript.

† National Research Council Fellow in Medicine, 1933-34.

‡ This sample of thyroglobulin was generously furnished by Dr. B. O. Barnes, of the Department of Physiology, University of Chicago; the protein was prepared from hog thyroids.

1 Barnes, B. O., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 680.

2 Heidelberger, M., and Palmer, W. W., *J. Biol. Chem.*, 1933, **101**, 433.

3 Oswald, A., *Z. physiol. Chem.*, 1899, **27**, 14; 1901, **32**, 121.

or Eckstein[4] found in their preparations. Barnes has shown, however, that the iodine content of thyroglobulin preparations varies with the locality from which the glands are obtained.[5]

TABLE I.
Some Analyses of Thyroglobulin.

	Analysis I	Analysis II	Aver.
	gm.	gm.	%
Basic Amino Acids			
Protein taken	37.7	29.9	
	%	%	
Histidine	0.65	0.58	0.62
S in diflavianate, theory 8.17	8.15	8.13	
Arginine	8.06	8.37	8.22
S in flavianate, theory 6.56	6.61	6.65	
Lysine	1.98	1.88	1.93
N in picrate, theory 18.67	18.59	18.58	
Total Nitrogen			15.58
" Sulfur			1.46
" Iodine§			0.75
" Phosphorus			0.08

§ We are indebted to Mr. F. Paquin, Jr., for the iodine analyses.

The analytical procedure followed for the estimation of the basic amino acids was that employed by Vickery and White[6] in a recent analysis of casein. The results of the determinations, expressed as percentages of the ash- and moisture-free protein, are shown in Table I together with the more important analytical data. The 2 analyses were conducted independently by the authors, and the close agreement indicates the accuracy with which it is possible to obtain duplicate results with the procedure as developed by Vickery and co-workers.

The results of the present analysis are expressed in Table II in terms of amino acid nitrogen as per cent of the total nitrogen. Eckstein's results[4] are included for purposes of comparison. The values for arginine are in good agreement, but there is no relation-

TABLE II.
Basic Amino Acids of Thyroglobulin, Expressed as Nitrogen in % of Protein Nitrogen.

	Analysis I	Analysis II	Aver.	Results of Eckstein[4]
Histidine N	1.13	1.00	1.07	11.92
				11.34
Arginine N	16.64	17.28	16.96	16.55
				16.27
Lysine N	2.44	2.31	2.38	4.43
				4.19

[4] Eckstein, H. C., *J. Biol. Chem.*, 1926, **67**, 601.

[5] Barnes, B. O., personal communication.

[6] Vickery, H. B., and White, A., *J. Biol. Chem.*, 1933, **103**, 413.

ship whatsoever between the results he has reported for histidine and our own. The high histidine value obtained by Eckstein with the Van Slyke distribution method was checked by him by means of the bromination technique of Plimmer and Phillips.[7] The close agreement between the results of these 2 methods was taken as evidence for the validity of the histidine figure. It is well known, however, that the Van Slyke method may yield values for histidine considerably greater than those found by the isolation technique, particularly in the presence of considerable amounts of cystine. This is strikingly illustrated by the published analyses of wool. Marston,[8] employing the method of Van Slyke, gave 6.9% as the amount of histidine yielded by this keratin; the modified silver precipitation procedure, however, indicates the presence of only 0.66%,[9] a value which has been checked by Stewart and Rimington.[10]

Plimmer and Phillips developed their method for application to pure histidine solutions. They likewise applied it to gelatin, this protein being selected because it is lacking in tyrosine, tryptophane and cystine, amino acids which markedly interfere with the accuracy of the histidine determination. These investigators clearly state, however, that "further work will be necessary to show that the method is applicable to other proteins, and to ascertain the effect of the presence of cystine." Data at present available in this laboratory indicate that thyroglobulin yields a considerable proportion of cystine. It is therefore probable that the histidine methods employed by Eckstein might yield misleading results when applied to this protein.

The lysine figure calculated from the weight of isolated pure lysine picrate is considerably lower than that obtained by Eckstein using the Van Slyke method. The magnitude of this difference between the results of the 2 investigations is of the order generally observed in a comparison of results of the determination of this amino acid by the 2 analytical procedures. Expressed as per cent nitrogen of the total nitrogen, the lysine yielded by edestin and casein, for example, is 2.25%[11] and 7.72%[6] respectively, by the isolation technique; the corresponding values by Van Slyke's method are 3.86%[12] and 10.3%.[13]

---

[7] Plimmer, R. H. A., and Phillips, H., *Biochem. J.*, 1924, **18**, 312.

[8] Marston, H. R., Council Sc. and Ind. Research, Commonwealth of Australia, 1928, Bull. 38.

[9] Vickery, H. B., and Block, R. J., *J. Biol. Chem.*, 1930, **86**, 107.

[10] Stewart, A. M., and Rimington, C., *Biochem. J.*, 1931, **25**, 2189.

[11] Vickery, H. B., and Leavenworth, C. S., *J. Biol. Chem.*, 1928, **76**, 707.

[12] Van Slyke, D. D., *J. Biol. Chem.*, 1911, **10**, 15.

[13] Van Slyke, D. D., *J. Biol. Chem.*, 1913, **16**, 531.

Summary. Analysis of the basic amino acids of thyroglobulin by direct isolation methods indicates that this protein yields 0.62% of histidine, 8.22% of arginine and 1.93% of lysine.

7678 P

Quantitative Studies with the Friedman Test in Excessive Vomiting of Pregnancy.*

FERDINAND J. SCHOENECK. (Introduced by Walter S. Root.)

From the Department of Obstetrics, Syracuse University, College of Medicine.

Using a modification of the technique of Friedman and Lapham,[1] involving intravenous injection of urine and the inspection of the ovaries after a period of 48 hours, an effort was made to determine the minimum amount of urine required to produce a positive reaction in cases of normal pregnancy. Observations were made between the fifth and the fourteenth weeks. All animals used were

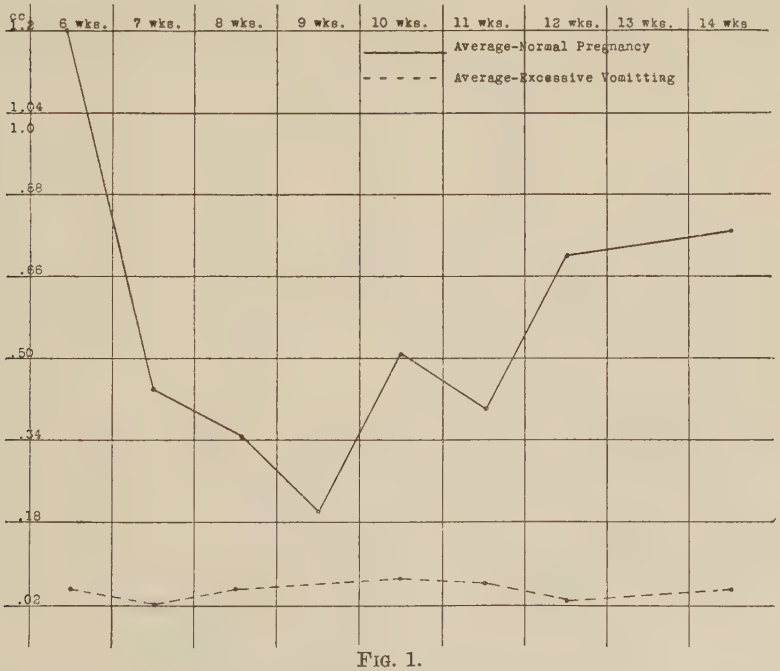


FIG. 1.

* Aided by a grant from the Hendricks Research Fund.

1 Friedman, M. H., and Lapham, M. E., *Am. J. Obst. and Gynec.*, 1931, **21**, 405.

virgin does between 3 and 4 months old and were obtained from the same source. One hundred and nineteen determinations were made on 31 pregnancies. Of 39 fractional tests, 2 were positive with .05 cc. of urine; 10 with .1 cc., and the remaining 27 required .5 cc. or more.

Sixty-two similar determinations were made on 10 cases of excessive vomiting, 4 of which were of the pernicious type. Of 16 tests on these patients, one, associated with a hydatidiform mole, was positive with .01 cc. and .0075 cc.; 4 were positive with .0125 cc.; 6 with .05 cc. and 4 with .1 cc.

Due consideration was given to the concentration of urine as evidenced by the specific gravity, since I[2] have previously shown that this is a factor in the amount of urine required to produce a positive reaction.

In 5 of the vomiting cases, followed to date, it was found that the amount of urine required to produce a positive reaction increased as the symptoms improved.

Although the amount of urine required to produce a positive reaction varies considerably in the normal cases, it would seem that the discrepancy between that group and the vomiting cases is too great to be explained by simple concentration of urine or normal variation.

7679 C

Sweat Secretion Produced by Pilocarpine in the Cat.*

J. C. HINSEY AND C. C. CUTTING.

From the Department of Anatomy, Stanford University.

Completely denervated sweat glands may be made to secrete by pilocarpine (Langley and Anderson,[1] Langley[2]), in spite of the fact that section of a peripheral nerve may lead to a much diminished secretion, which Burn[3] attributed to the section of the somatic motor fibers rather than to the sensory or sympathetic fibers in the

[2] Schoeneck, F. J., *Am. J. Obst. and Gynec.*, 1932, **23**, 712.

* This study was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

[1] Langley, J. N., and Anderson, H. K., *J. Physiol.*, 1904, **31**, 423.

[2] Langley, J. N., *J. Physiol.*, 1922, **56**, 110.

[3] Burn, J. H., *J. Physiol.*, 1925, **60**, 365.

nerve. Burn believed that this was due to circulatory effects produced by muscular inactivity and found that it paralleled the decreased vasodilator response to histamine. Furthermore he found that pilocarpine following deafferentation of the hind limb in the cat produced a diminished response, accompanying the decreased movement and impaired circulation that was present for a time following the operation. This diminution disappeared later on. Burn,[3, 4] further showed that the exaggerated sweat response following sympathectomy was due mainly to the removal of the sympathetic control of the blood vessels and also in some animals to a hypersensitiveness of the denervated glands and vessels to pilocarpine.

Guttmann[5] has reviewed the literature on this problem of sweat secretion in man and has described it as seen in a number of Foerster's patients. He has suggested that the sweat glands have a secretory innervation through the sympathetic chain as well as directly by way of the ventral roots to the spinal nerves, and an inhibitory innervation by way of the dorsal roots (Dieden[6]). Langley[7] denied vigorously that there could be any direct secretory innervation to sweat glands aside from that passing through the sympathetic chain in the cat. He also found a facilitory instead of an inhibitory effect in the cat on stimulation of the 6th and 7th lumbar dorsal roots in case the secretion was already in progress, due, he thought, to the presence of the accompanying dorsal root vasodilatation.

In the case of the human, sweating plays a much more important rôle in the control of body temperature, and the distribution of the sweat glands is much more extensive. However, it would seem strange that there should be wide differences in the distribution of visceral innervation in man and in the experimental animals, in which the only secretory innervation known is by way of the thoracolumbar sympathetic, and no inhibitory innervation by dorsal root fibers has been demonstrated.

In our laboratory we have 4 cats in which the right hind limb has been deafferented for 3 years and sympathectomized for 2 years, 2 animals which have been sympathectomized for 3 years and deafferented for 2 years, and 2 which have been sympathectomized 20 and 22 months respectively. Kennard and Fulton[8] have observed a

[4] Burn, J. H., *J. Physiol.*, 1922, **56**, 232.

[5] Guttmann, L., *Ztschr. f. d. ges. Neurol. u. Psychiat.*, 1931, **135**, 1.

[6] Dieden, H., *Ztschr. f. Biol.*, 1916, **66**, 387.

[7] Langley, J. N., *J. Physiol.*, 1891, **12**, 347.

[8] Kennard, M. A., and Fulton, J. F., personal communication, 1934.

difference in the sweating produced by pilocarpine injected into monkeys in which the premotor area (area 6 of Brodmann) had been unilaterally removed. There was no difference in the sweating produced by pilocarpine following unilateral deafferentation in the monkey. At Fulton's suggestion, we have made some observations on sweating in our series of cats. The sympathectomies consisted of removing the right abdominal sympathetic chain from L2 through L7 and in a number of cases through S1. The deafferentation consisted of cutting the right dorsal roots L4-S2 proximal to the dorsal root ganglia. The somatic motor innervation was still intact in these animals; in fact they walked quite well on the dorsum of the foot in the deafferented limb.

Pilocarpine HCl (5 mg. per kg.) was injected subcutaneously and the pads of the feet were observed for sweating. Care was taken to remove any superficial horny layers of epidermis which had accumulated on the pads of the operated side. The observations were all qualitative and not quantitative. Sweating appeared at about the same time (3 to 5 minutes) on the operated side as on the normal side and seemed to be about the same in amount (observed with hand lens). This was true not only for the 6 deafferented-sympathectomized cats but also for the 2 in which the sympathetic chain alone was removed. Thus it would seem that sweating can take place 2 years after all the known pathways to the skin were interrupted. Furthermore, 20 and 22 months after sympathectomy no increased secretion was seen on the operated side.

These observations demonstrate that 2 years after denervation of the skin, the sweat glands had not atrophied appreciably in spite of their separation from the central nervous system. Furthermore they support Burn's contention that the decreased response to pilocarpine following section of peripheral nerves is due to section of somatic motor fibers which were still intact in our experiments. Inasmuch as in the cat there is no known direct secretory innervation by way of the ventral roots and spinal nerves, the effect of somatic motor impulses must be indirect through their action on skeletal muscles. The preganglionic sympathetic supply to the right hind limb had been completely removed by the abdominal sympathectomy. However, in all of these experiments the 2S ganglion was left in so that a few postganglionic fibers were still present in the foot-pads (Langley[7]). This supply, however, is very slight and it does not seem probable that the sweat which was seen in the foot-pads of the operated side could be attributed to an action of the pilocarpine upon the nerve endings of these fibers. It is more likely that the action was directly upon the gland cells as Langley[2] believed.

7680 C

A Simple Apparatus for Metabolic Measurements on Small Animals.*

M. L. TAINTER AND DAVID A. RYTAND.

From the Departments of Pharmacology and of Medicine, Stanford University School of Medicine, San Francisco.

In view of the highly complicated apparatus which is usually described as being necessary to measure the oxygen consumption of the smaller laboratory animals, it appears worthwhile to point out that equally valuable and accurate results may be obtained with an extremely simple device. The apparatus described herein seems so obvious a method of measuring metabolism by the use of oxygen consumption that there is hardly anything original in its design.

In essence the apparatus consists of a thin-walled copper tank, inside of which (K) an animal such as a frog, rat, mouse or guinea pig may be placed. The animal sits on a floor (L) of coarse wire gauze (3 mesh) supported on legs above the surface of a fine wire gauze basket (M) which contains 12 mesh soda-lime. This raised floor permits the feces to drop out of the way so the animals do not soil themselves and spend time cleaning their fur. A second basket for soda lime (G) is suspended in the top of the tank. This basket is made double so the animals cannot get their noses against the soda-lime and sniff the irritating dust up their nostrils. A perfect seal is easily obtained by having a flat top (D) with a downward projecting rim (E) which extends into a moat (F) containing oil or water. This gives a perfectly tight liquid seal without the necessity of having the usual ground joints or clamps, etc. Through the top projects the tube (C) of an ordinary float volume recorder (B). Changes in volume are recorded from the level of the volume recorder on the scale (A). By having the scale drawn or pasted on a mirror, parallax in the readings is avoided; a thermometer (P) also projects through the top. Oxygen is admitted to the tank through a copper tube (O) in the bottom, the flow being controlled by a stopcock. A weight (N) is fastened to the bottom to prevent the tank floating when immersed in a water bath (J). A hole is cut in the side of the tank over which is sealed with sealing wax, a glass plate (I) which serves as a window for observing the activity of the animal.

* Supported in part by a grant from the Rockefeller Fluid Research Fund of the School of Medicine, Stanford University.

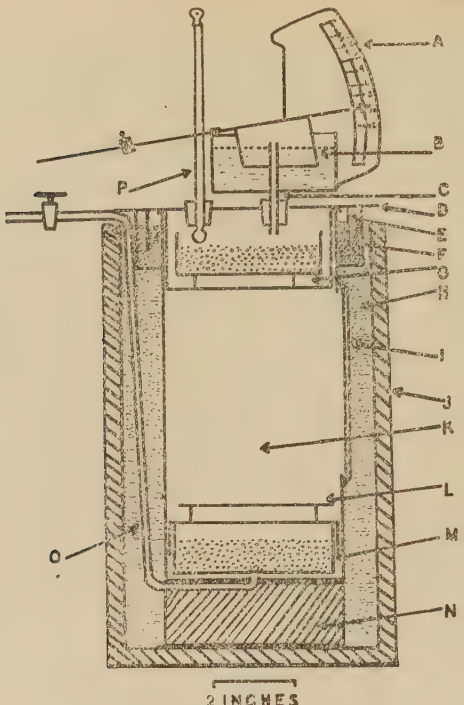


FIG. 1.

The apparatus (Fig. 1) is large enough for rats or guinea pigs of at least 600 gm. in weight. The reason for the accuracy of this apparatus is that it has a negligible excess of air space. This minimizes the change in absolute volume of the gas inside if any slight fluctuations occur in the temperature. The heat capacity of the water jacket (H) is sufficient to hold the temperature very constant, even when the animal has metabolic rates several hundred per cent above the normal, as during dinitrophenol poisoning. Under conditions of prolonged observations or extreme atmospheric conditions, the apparatus can be placed in a constant temperature bath, or water from such a bath may be allowed to run through the glass jar.

In actual practise the animal is put into the tank and permitted to relax for 10 or more minutes, until there are no movements over a reasonable period of time. Meanwhile oxygen slowly runs into the tank from an ordinary gas cylinder at a rate slightly faster than the utilization, the excess bubbling out the top by upward displacement of the float recorder (B). When a measurement is to be made, the oxygen inflow is stopped by turning the stop-cock, and

the float recorder drops down across the scale as the oxygen is used up. The time required to use from 2 to 8 cc. of oxygen is measured with a stopwatch. A series of readings are taken while the animal is quiet. The readings are accepted if there is no progressive drift in a series of 5 or more consecutive readings taken over a period of 5 to 10 minutes. These values should check each other within a maximum range of not more than 10%.

The experience of several years' steady use of this apparatus shows that no difficulty is encountered in obtaining values in good accord with those of more complicated instruments. Reference to the recently published papers on the metabolic stimulation caused by dinitrophenol will show the type of data that has been obtained with this apparatus.[1] The apparatus may be made smaller for animals below 150 gm. The one illustrated in this paper, and one about half the size, has served all needs for animals between the weights of 30 and 600 gm.

Summary. A simple apparatus is described for measuring the metabolic rates of smaller laboratory animals. It requires no unusual materials and can be made in a few hours by anyone able to solder. Its accuracy and simplicity are derived from the fact that by keeping the size down to a minimum, the contained gas volume is very small and the necessity for rigorous temperature control and circulation of the air is thereby eliminated. The values reported in the literature, with more complicated apparatus and groups of animals measured over longer intervals of time, are reproducible in this simple apparatus using single animals and observation-periods of relatively few minutes.

7681 C

Conventional Bacteriologic Technics in "Hormone" and "Vitamine" Research.*

EDWARD E. DART. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

A fairly uniform bacteriologic technic is in current use for the demonstration of "hormones," "vitamines" and "accessory growth-

[1] Tainter, M. L., *J. Pharm. Exp. Therap.*, 1934, **51**, 45.

* Work supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

factors." As a typical example of this technic, Nicolle and Césair[1] inoculated 10% broth-dilutions of specific immune serum with homologous bacteria, and invariably obtained a much more luxuriant growth in 10% homologous anti-serum than in normal or hetero-immune serum controls. From this they concluded that the specific "antibodies" in question are in reality specific "growth-stimulating hormones" for the corresponding bacteria.

In technics of this type there are numerous arbitrary experimental constants which may conceivably condition the end-result. Prominent among these arbitrary constants are the dosage, age, growth-phase and nutritional condition of the inoculum, and the time and method of reading the end-result. Most "hormone" data, for example, are recorded in terms of relative turbidity at the end of 24-hours incubation.

In order to test the reliability of this arbitrary time-factor, parallel "viable" and "total" population growth-curves were plotted for *Streptococcus hemolyticus* in 100 cc. beef-infusion broth and broth containing 0.1% beet juice. Typical curves thus obtained are recorded in Figure 1.

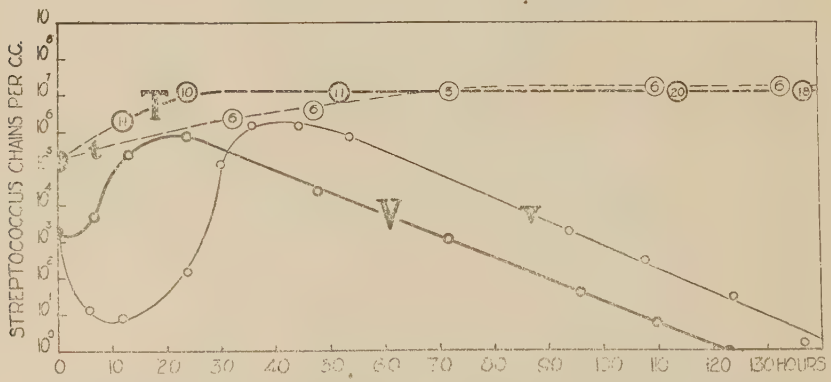


FIG. 1. Effects of Beet "Auximone" on Streptococci.
100 cc. beef-infusion broth plus 0.3 cc. 24-hour veal-infusion broth culture of *S. hemolyticus*. Total population counts (t, Spencer counting chamber) recorded in terms of chains per cc., average number of cocci per chain being shown in the recorded circles. Viable population counts (v) obtained by quantitative dilution methods, veal-infusion agar, 4-day plate-count chosen as the "viability" index. T, V: parallel "total" and "viable" population counts in beef-infusion broth plus 0.1% beet-juice (Berkefeld filtrate).

From these curves, it is seen that the conventional selection of the 24-hour turbidity (or "total" population count) as the recorded reading gives data apparently proving that beet-juice contains a powerful growth-stimulating hormone for streptococci. An arbi-

[1] Nicolle, M., and Césair, E., *Ann. Inst. Pasteur*, 1926, **40**, 43.

trary selection of the 2 to 5-day "viable" count, however, would give equally convincing evidence that beet auximone is a fairly efficient surgical antiseptic, causing premature death of 99% of the potentially "viable" streptococci.

The curves as a whole could be explained with equal plausibility, however, by assuming that 0.1% beet-juice is without direct effect upon S. *hemolyticus,* its sole indirect action being to neutralize certain unknown toxic factors in beef-infusion broth, thus shortening the usual "lag phase" in population-growth. Otherwise the 2 sets of growth-curves are practically identical.

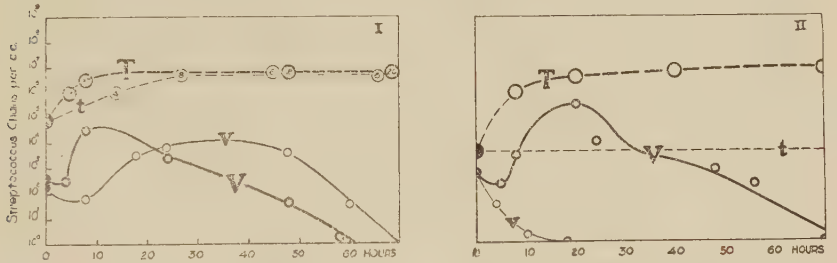


FIG. 2. "Logarithmic Phase" vs. "Lag Phase" Streptococci.
Technic as in Fig. 1, except for the use of heat-sterilized beet-juice (100°C., 30 min.).
I. "Logarithmic phase" inoculum (24-hour growth in veal-infusion broth.)
II. "Lag phase" inoculum (10-hour growth in veal-infusion broth).

With streptococcus inoculum in the "lag phase" instead of the "logarithmic phase" of growth, the superimposed second "lag phase" on transfer to beef-infusion broth often leads to a fairly rapid "death" of the inoculated organisms. (II, Fig. 2). The prevention of this second "lag phase" in the relatively non-toxic beet-juice-broth, therefore, gives data apparently proving that beet-juice contains a "vitamine" absolutely essential for the life of *S. hemolyticus.* Here, tested under identical conditions, 0.1% beet-juice shortens the life of a vigorous ("logarithmic phase") inoculum, but prolongs the life of a less vigorous ("lag phase") transplant.

Paradoxes and inconsistencies of this type strongly suggest that conventional bacteriologic technics are not always reliable sources of information in "vitamine" and "hormone" research.

7682 C

Normal Variations in the Susceptibility of Human Fibrin to Streptococcus Fibrinolysin.*

J. K. VAN DEVENTER. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

There are marked differences in the susceptibilities of different samples of presumably normal human plasma-clot to the thrombolysin formed or secreted by certain strains of *S. hemolyticus.* While one clot may be completely liquefied within 30 minutes after admixture with a given lytic culture, the clot from a second, presumably normal individual may show no sign of softening even after 24-hours incubation with this culture.[1]

To test the possibility that this difference may be due to quantitative differences in fibrin-content, parallel fibrinolytic titrations were made with different concentrations of the same isolated fibrin. Typical data thus obtained are recorded in Table I.

TABLE I.

Quantitative Variations in Fibrin-Content.

Human fibrinogen and thrombin isolated by the Tillett-Garner technic[2] and redissolved in such concentrations as to produce serum-free clots having 1-fold, 2-fold and 4-fold the fibrin-content of the average normal plasma, quantities and dilutions being otherwise the same as that adopted by Tillett and Garner. Fibrinolytic titration made throughout with the same lytic filtrate.

+, complete liquefaction of the serum-free fibrin-clot at the time of observation; ±, partial liquefaction; 0, no demonstrable softening.

Dilution of fibrinolytic filtrate	The same fibrinogen-thrombin complex tested in:											
	1-fold concentration				2-fold concentration				4-fold concentration			
	10 min.	30 min.	1 hr.	2 hr.	10 min.	30 min.	1 hr.	2 hr.	10 min.	30 min.	1 hr.	2 hr.
1:5	+	+	+	+	0	+	+	+	0	+	+	+
1:10	+	+	+	+	0	+	+	+	0	+	+	+
1:50	0	+	+	+	0	±	+	+	0	0	+	+
1:100	0	0	+	+	0	0	0	+	0	0	0	±
1:200	0	0	±	+	0	0	0	0	0	0	0	0
Control (autolytic test)	0	0	0	±	0	0	0	0	0	0	0	0

From this table it is seen that the rate and completeness of fibrinolysis with a given lytic filtrate varies inversely with the fibrin-content. Complete insusceptibility to lysis, however, does not re-

* Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Van Deventer, J. K., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 50.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

sult from an increase in fibrin-content even to 4 times that of the average normal human plasma-clot. This result is in line with the statistical evidence reported by Hadfield, Magee and Perry.[3] These investigators found no correlation between fibrin-content and fibrinolytic susceptibility in different blood samples.

To test the possibility that the observed differences in susceptibility may be due to qualitative differences between "normal" and "immune" fibrins, parallel fibrinolytic titrations were made with fibrins isolated from susceptible and resistant plasma-clots. A typical comparison of this type is recorded in Table II.

TABLE II.
Qualitative Variations in Fibrin-Content.
Serum-free fibrins from susceptible and resistant plasmas titrated against the same fibrinolytic filtrate.

Dilution of fibrinolytic filtrate	Fibrin from susceptible plasma				Fibrin from resistant plasma				Susceptible fibrinogen reisolated from resistant serum			
	10 min.	30 min.	1 hr.	2 hr.	10 min.	30 min.	1 hr.	2 hr.	10 min.	30 min.	1 hr.	2 hr.
1:1	+	+	+	+	±	+	+	+	+	+	+	+
1:2	+	+	+	+	0	+	+	+	0	+	+	+
1:4	+	+	+	+	0	+	+	+	0	+	+	+
1:8	+	+	+	+	0	+	+	+	0	+	+	+
1:16	+	±	+	+	0	0	+	+	0	0	+	+
1:32	0	0	±	+	0	0	0	±	0	0	0	±
Control (autolytic test)	0	0	0	0	0	0	0	0	0	0	0	0

From this table it is seen that serum-free fibrins isolated from resistant and susceptible plasmas are very nearly identical in their fibrinolytic susceptibilities. The slightly delayed softening of the "immune" fibrin is presumably due to adsorbed antibodies. This is confirmed by the fact that susceptible fibrinogen redissolved in and afterwards reisolated from resistant serum, has approximately this same slight degree of insusceptibility. This conclusion is in line with data reported by Fuchs[4] in his study of "normal" and "immune" fibrins by other technical methods.

Variations in plasma-clot susceptibility, therefore, are apparently due solely to qualitative or quantitative variations in anti-fibrinolytic serum factors. To test this conclusion, parallel antifibrinolytic titrations were made with serums isolated from susceptible and resistant plasma-clots. A typical titration of this type is recorded in Table III.

From this table it is seen that the serum from the most susceptible normal human plasma thus far tested by us contains an "antibody"

[3] Hadfield, G., Magee, V., and Perry, C. B., *Lancet*, 1934, **226**, 834.

[4] Fuchs, H. J., *Z. f. Immunitatsfossch.*, 1928, **57**, 320.

TABLE III.

Antifibrinolytic Titer of Susceptible and Resistant Serums.

An arbitrary fibrinolytic unit was adopted, equal to approximately three times the minimum 30-minute lytic unit for serum-free fibrin. This arbitrary lytic unit was mixed with serial dilutions of susceptible and resistant serum, the mixtures incubated 30 minutes, then tested against the same serum-free fibrinogen-thrombin complex.

Dilution of neutralizing serum	Serum from susceptible plasma-clot				Serum from resistant plasma-clot			
	10 min.	30 min.	1 hr.	2 hr.	10 min.	30 min.	1 hr.	2 hr.
1:3	0	0	0	0	0	0	0	0
1:9	0	0	0	0	0	0	0	0
1:27	0	+	+	+	0	0	0	0
1:81	+	+	+	+	0	0	0	±
1:243	+	+	+	+	0	0	+	+
Control (no antiserum)	+	+	+	+	+	+	+	+

capable of neutralizing our arbitrary fibrinolytic unit in dilutions as high as 1:9. Serum from our most resistant plasma neutralizes the same lytic unit in dilutions as high as 1:81.

7683 C

Dorsal Roots of Spinal Nerves and Regulation of Skin Temperature.*

K. B. CORBIN AND J. C. HINSEY.

From the Department of Anatomy, Stanford University.

Stricker[1] first showed that stimulation of the dorsal roots of spinal nerves produced a vasodilatation in peripheral blood vessels. This observation has been confirmed by Bayliss,[2] Ranson and Wightman[3] and others. Hinsey and Gasser[4] showed that the fibers mediating vasodilatation on stimulation of the dorsal roots were ones whose potentials were found in the C-spike of the action potential. Vasodilator reflexes over the dorsal roots were demonstrated in positive experiments by Bayliss[5] and Fofanov and Tschalussow.[6] Recently

* This study was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

[1] Stricker, M., *Sitzber. d. k. Akad. d. Wiss., Wien Math. nat. Kl.*, 1876, **74**, 173.

[2] Bayliss, W. M., *J. Physiol.*, 1901, **26**, 173.

[3] Ranson, S. W., and Wightman, W. D., *Am. J. Physiol.*, 1922, **62**, 392.

[4] Hinsey, J. C., and Gasser, H. S., *Am. J. Physiol.*, 1930, **92**, 679.

[5] Bayliss, W. M., *J. Physiol.*, 1902, **28**, 276.

[6] Fofanov, L. L., and Tschalussow, M. A., *Pflüger's Arch.*, 1913, **151**, 543.

Bishop, Heinbecker and O'Leary[7] and Zuckerman and Ruch[8] have again obtained positive results which tend to show that the vasodilator fibers in the dorsal roots are accessible to reflex stimulation.

The observations of Hinsey and Cutting,[9] Hinsey,[10] and of Zuckerman and Ruch[8] showed that the skin temperatures in deafferented hind limbs were lower than in the opposite normally innervated limbs. These observations could either be attributed to an overaction of the vasoconstrictor pathways or to an absence of a dorsal root vasodilator mechanism in the colder limb. Zuckerman and Ruch state that when a limb is connected only with the spinal dorsal roots, it shows a different behavior than the opposite completely denervated limb (method of denervation is not stated; unless a bilateral abdominal sympathectomy was performed in addition to cutting the lumbosacral spinal roots, the denervation would not have been complete). The former fell in temperature much less than the denervated limb and usually less than the normally innervated skin. Hinsey observed that when a deafferented limb was sympathectomized, it became warmer than the opposite normally innervated limb.

The experiments here reported were carried out on 4 young adult cats. In the first operation, the abdominal sympathetic trunks were removed bilaterally from 2L-7L inclusive. By this operation, the vasoconstrictor pathways to both hind limbs were broken. After about 4 weeks, the right hind limb was deafferented by cutting the dorsal roots of 4L-2S inclusive. Thus the right hind limb was connected with the spinal cord only by ventral root somatic motor fibers while the left limb was supplied with both somatic sensory and motor fibers. If the dorsal roots are mediating efferent impulses to the vessels of the skin, the right hind limb should show a difference under certain conditions as compared to the opposite limb. Skin temperatures from both of the fore limbs and the hind limbs were recorded with a thermocouple. The animals were subjected to different room temperatures from 6 to 30°C. The skin temperatures were recorded immediately after placing the animals in the different room temperatures and after they had been there from 10 to 60 minutes. In these 4 animals which we have followed for 4 weeks, we have no indication whatsoever that there is any significant difference between the skin temperatures in the 2 hind limbs. This state-

[7] Bishop, G. H., Heinbecker, P., and O'Leary, J. L., *Am. J. Physiol.*, 1933, **106**, 647.

[8] Zuckerman, S., and Ruch, T. C., *Am. J. Physiol.*, 1934, **109**, 116.

[9] Hinsey, J. C., and Cutting, C. C., *Anat. Rec.*, 1932, **52**, 57.

[10] Hinsey, J. C., *Am. J. Physiol.*, 1934, **109**, 53.

ment holds for all the room temperatures used and is true immediately after the room temperature is changed and after a period for adaptation has elapsed. The maximal differences between the fore and hind limbs occurred at room temperatures between 16° and 20°C. and tended to decrease as the temperature became colder or warmer. After a period of 30 minutes in a room temperature of 30°C., the temperatures in the fore and hind limbs were essentially equal (confirmation of Reichert[11]).

These experiments do not support a reflex control of the blood vessels of the skin *via* the dorsal roots, as evidenced by skin temperature in the cat. With the thoracolumbar sympathetic innervation bilaterally removed, unilateral section of the dorsal roots does not produce a difference in the skin temperatures on the 2 sides under the experimental conditions we have used. We believe that the lower skin temperatures which Hinsey reported for deafferented limbs are to be attributed to an over-action of the vasoconstrictor mechanism in the absence of some inhibitory control from the somatic afferent supply to the respective limbs. We realize that, if there are afferent neurones situated in the sacral chain ganglia such as Schwartz[12] has reported in the stellate and lumbosacral chain ganglia of the cat, they were not removed in our experiments. If the vasomotor effects which Rosenblueth and Cannon[13] have described in sympathectomized animals are due to dorsal root efferent conduction, they were not reflected in the skin temperatures of the foot-pads as we recorded them.

7684 C

Concerning Gonadotropic Substances in Mare Serum.

H. H. COLE AND G. H. HART.

From the College of Agriculture, University of California, Davis.

Evans *et al.*[1] have shown that there is a wide distribution of a substance in the urine and blood serum of mammals which resembles prolan, in that when it is mixed *in vitro* with the pituitary synergist the effect upon the ovary of the immature rat is enhanced. Evidence

[11] Reichert, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 473.

[12] Schwartz, H. G., *Am. J. Physiol.*, 1934, **109**, 593.

[13] Rosenblueth, A., and Cannon, W. B., *Am. J. Physiol.*, 1934, **108**, 599.

[1] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

that a similar prolan-like substance is present in the blood serum of the non-pregnant and pregnant mare is given in Table 1.* Further the data indicate that the prolan-like substance is distinct from the substance in the blood serum of the pregnant mare which is so highly effective in stimulating the gonads when given alone. Following our nomenclature in previous publications we will term the latter substance the gonad-stimulating hormone.

TABLE I.

Response of Immature Rats to Mare Serum Alone and to the Combination of Mare Serum and Pituitary Synergist. The Rat Dose of Synergist Was in Every Instance 2 mg.

Serum taken after infertile mating days	Serum	Serum alone			Serum plus synergist		
		Serum injected	No. of rats	Av. wt. of 2 ovaries	Serum injected	No. of rats	Av. wt. of 2 ovaries
		cc.		mg.	cc.		mg.
8	N1	10	4	17*	2	12	26‡
11	N7	—	—	—	2	6	37‡
16	N2	10	4	18*	2	6	42‡
23	N3	10	4	19*	2	6	49‡
31	N4	10	4	19*	2	6	42‡
37	N5	10	4	19*	2	6	26‡
41	N6	10	4	14*	2	6	42‡
Average for sera of non-pregnant mares				18*			36‡
175 days pregnant	P1	2	6	21†	2	6	35‡
165 " "	P2	2	6	23‡	2	6	37‡
Average for sera of mares at mid-pregnancy				22			36‡
80 days pregnant	7	0.04	6	22‡	0.04	6	21‡
80 " "	7	0.08	6	24‡	0.08	12	37‡
80 " "	8	0.08	6	25‡	0.08	6	25‡
Average for sera of mares in early pregnancy				24‡			28‡

*Ovaries of all rats in the group were infantile.
†Ovaries of 3 of the 6 rats had ripe follicles or corpora.
‡Ovaries of all rats in the group contained ripe follicles or corpora.

Referring to Table 1 it may be seen that equivalent amounts of non-pregnant and mid-pregnancy mare serum are equally effective in stimulating the gonads of immature rats when combined with the pituitary synergist. The amount of gonad-stimulating hormone present in the 2 types of sera differs greatly. Two cc. of the mid-pregnancy serum when given alone induces follicular development and luteinization whereas 10 cc. of the non-pregnant serum produces no response. In fact, we have shown previously that doses of 60 cc. of non-pregnant serum are without response on the immature rat. Thus it is clear that there is no relationship between the response

* The synergist was prepared and administered according to the method of Evans *et al.*[2] The average ovarian weight of 11 rats receiving 2 mg. of synergist alone was 22 mg.

[2] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

of the serum when given alone and the response of serum plus synergist. This fact is further demonstrated when the response of early pregnancy blood serum highly potent in gonad-stimulating hormone is compared to non-pregnant serum. The amount of early pregnancy serum injected contained 2 to 4 rat units of gonad-stimulating hormone while the non-pregnant serum contained none. Still the response of the early pregnancy serum plus synergist was less than the non-pregnant serum plus synergist. These results appear to us to be most easily explained by assuming that non-pregnant mare serum contains a single recognizable gonadotropic substance which is only effective when mixed *in vitro* with pituitary synergist and that pregnancy serum contains 2 substances—one effective by itself or possibly in conjunction with the pituitary of the recipient —the other effective only when mixed *in vitro* with the pituitary synergist.

Table 1 shows an apparent correlation between the time following an infertile mating and the response elicited in the ovaries of immature rats by the serum when combined with synergist. Further tests would have to be made to demonstrate that the increasing ovarian response is related to pseudo-pregnancy which itself has not yet been demonstrated in the mare.

We have made no attempt to determine the identity of these substances in mare serum with gonadotropic substances described by other workers. Hisaw and his associates have held the view, which has become increasingly popular, that the pituitary contains two gonadotropic substances, a "follicle-stimulating" and a "luteinizing" substance. They[3] were the first to give conclusive proof that the pituitary contains 2 gonadotropic substances. Our results presented above could be explained by assuming that the "luteinizing" substance is present both in non-pregnant and in pregnant mare serum and the "follicle-stimulator" only in the pregnant serum. In fact, Hisaw and his associates (personal communication) report that the serum of the pregnant mare contains these 2 substances. It seems very probable, however, that the recent finding of Smith *et al.*[4] in regard to the remarkable specificity of follicle-stimulating urine upon the seminiferous tubules of the hypophysectomized male rat and similar findings of Evans *et al.*[5] in regard to the hypophyseal

[3] Fevold, H. L., Hisaw, F. L., and Leonard, S. L., *Am. J. Physiol.*, 1931, **97**, 291.

[4] Smith, P. E., Engle, E. T., and Tyndale, H. H., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 745.

[5] Evans, H. M., Pencharz, R. I., and Simpson, M. E., *Endocrinology*, 1934, **18**, 607.

synergist may lead to a modification of views pertaining to the nature of the gonadotropic hormones.

7685 C

Hemolysins to which Reticulocytes are Especially Vulnerable in the Plasma of Primary Anemia.

WILLIAM DOCK AND CAMILLE MERMOD.

From the Department of Medicine, Stanford University Medical School, San Francisco.

Ucko[1] has described a saponin-like substance present in normal plasma but in much larger amounts in active cases of primary anemia. It is more toxic to the reticulocytes than to the mature cells. It seemed to us that the presence of such a substance, rather than a structural defect in the reticulocytes of patients with primary anemia, might explain the fall of 30-60% in the reticulocyte count on incubating sterile defibrinated blood of active cases of primary anemia.[2] Blood from normal persons, from secondary anemia cases, or from cases of primary anemia during remission does not have a fall in reticulocyte count when incubated in the same way.[2, 3]

We therefore took blood from 4 active cases of primary anemia just before starting intensive parenteral liver therapy and preserved the plasma on ice for 4-7 days. Washed cells obtained at the height of the reticulocyte rise were then mixed with twice their volume of fresh plasma, and also, in the same proportion, with plasma obtained before treatment. These tubes were incubated at 37° C. All the blood was handled with sterile technique and counts were made only if there was no bacterial growth on incubation.

In one case, the mildest and least jaundiced (Van den Bergh, indirect, 1.25 units), the blood taken during the active stage had to be transported and cells and plasma were not separated until several hours after being drawn. This patient had 2.4 million red cells per cu. mm. before treatment and in this case the reticulocyte count in the tubes containing pre- and post-treatment plasma was the same after 24 hours incubation. The other 3 patients all had counts under 1.4 million and indirect Van den Berghs of 1.5 to 3 units before

[1] Ucko, H., *Z. f. klin. Med.*, 1931, **118**, 22.

[2] Buckman, T. E., and MacNaugher, E., *J. Med. Research*, 1923, **44**, 61.

[3] Mermod, C., and Dock, W., *Arch. Int. Med.*, in press.

treatment. At the time the reticulocyte peak was reached the Van den Berghs had fallen to 0.6 to 0.8 units. In all of these the reticulocyte count fell on incubating the cells in plasma taken before treatment, but not in that taken during the period of high reticulocytosis. In the first 2 cases the proportion of cells to plasma was as 1 to 2 during incubation; in the third case a 1 to 10 suspension was also observed and showed a more striking fall in reticulocyte count in the plasma taken during the acute stage (Table 1).

TABLE I.

The reticulocyte percentage after incubation for 24 hrs. at 37° C. of cells obtained during remission in plasma obtained during remission (Plasma B) and that obtained before treatment (Plasma A). In case P, Plasma A obtained on March 8, plasma B on March 12; in case S, A on April 2, B on April 9; in case M, A on September 11, B on September 16. Counts in plasma B were never lower than in fresh blood unless incubated 30-50 hours.

Reticulocyte Counts After Incubation.

	Case P	Case S	Case M	Case M.*
Plasma A	24	25	19	6.5
Plasma B	29	34	26	28

*Ratio of washed cells to plasma in these tubes 1:10, in all others 1:2.

This phenomenon can not be explained by increased reticulocyte resistance due to treatment, but suggests the presence of a hemolysin, to which reticulocytes especially are vulnerable, in the cases of active primary anemia, and the disappearance of this substance during remission.

7686 C

Absence of Vasoconstrictor Substance in Blood of Rats with Renal Hypertension.

WILLIAM DOCK AND DAVID A. RYTAND.

From the Department of Medicine, Stanford University Medical School, San Francisco.

Using an adaptation of Griffith's and Collins' method for measuring systolic pressure[1] in rats which had developed hypertension as a result of renal insufficiency,[2] we have studied the effect of destroying the central nervous system on the blood pressure of normal and of hypertensive rats. The blood pressure was determined under light ether anesthesia, and then, after inserting a tracheal cannula con-

[1] Griffith, J. Q., Jr., and Collins, L. H., Jr., *Am. Heart J.*, 1933, **8**, 671.

[2] Chanutin, A., and Ferris, E. B., Jr., *Arch. Int. Med.*, 1932, **49**, 767.

nected with a device for arterial respiration, the rat was pithed and decerebrated. A cord, passed around the neck under the skin and trachea, was drawn tight crushing the cervical spine and ligating the carotid and vertebral vessels. Through a thoracic laminectomy a wire with a beaded tip was passed up and down the spinal canal. In this way the central nervous system can be destroyed quickly and with little blood-loss, and the preparation, without artificial warming, will last for 5 hours. The blood pressure remains constant during the first half hour, falling slowly thereafter. We followed the pressure during the period from 2 to 30 min. after pithing and decerebration in 10 normal and in 10 hypertensive male rats. The control level in the former was 117.6 mm. Hg average, varying from 85 to 136 mm.; in the latter the preoperative range was 151 to 209 mm., average 176.4 mm. After destroying the central nervous system the average level in the normals was 55.3 mm. Hg., range 42 to 66 mm., while the hypertensive group averaged 54.5 mm., range 45 to 66 mm. In the animals with destruction of the nervous system epinephrine and pituitrin, given intraperitoneally, caused a marked but transient rise in pressure. We therefore conclude that whatever pressor substance may be present in the plasma of rats with renal hypertension has no vasoconstrictor effect but acts through the vasomotor center. In some instances the initial pressure determinations after pithing were made within 2 minutes after beginning the denervation, and the fall of pressure was immediate and not gradual as would have been the case from cutting off the inflow of such pressor substances as epinephrine or pituitrin.

7687 P

Influence of Intermedin on Growth of Mouse Melanoma.

KANEMATSU SUGIURA.

From the Huntington Fund for Cancer Research, Memorial Hospital, New York.

It has been reported[1] that repeated injections of aqueous extracts prepared from fresh whole pituitary glands (sheep and cattle) or from fresh anterior lobes, had a slight but distinct stimulating effect upon the growth of the Passey mouse melanoma. With the Flexner-Jobling rat carcinoma, the Bashford mouse carcinoma 63, and the Rous chicken sarcoma, no stimulating action was observed.

[1] Sugiura, K., and Benedict, S. R., *Am. J. Cancer*, 1933, **18**, 583.

It was further shown in this institution that the aqueous extracts of the Passey mouse melanoma gave a distinct intermedin reaction on a small fish, called the Elritze (*Phoxinus laevis*).* On the other hand, aqueous extracts of the Flexner-Jobling rat carcinoma, the Sugiura rat sarcoma, the Heiman rat fibroadenoma 308, and the Rous chicken sarcoma gave either a negative or a very weak color reaction.

These experiments suggest that the melanoma-stimulating substance found in pituitary extracts and the intermedin-like substance present in melanoma tissue might be identical.

Since in preparing the extracts of the pituitary, both the anterior and middle lobes were used, there is a good reason to suppose that the hormone of the intermediary lobe of the hypophysis may possess much greater melanoma-stimulating action than that of the anterior pituitary. The following experiments were carried out with the object of testing this supposition.

Twenty young adult albino mice bearing 7-day-old Passey mouse melanoma were each injected subcutaneously with 0.05, 0.1, 0.2, or 0.3 cc. of intermedin† 3 times a week, over a period of 78 days. Each cubic centimeter contained from 2000 to 2500 fish units. The intermedin injections seemed to have no ill effect on the general health of these animals.

The results showed that the repeated injections of intermedin had no regressing influence upon the growth of transplanted melanomas. However, it had a slight but distinct stimulating effect upon the growth of this tumor. This action was most noticeable in the case of tumor-bearing animals receiving a daily dose of 0.1 cc. of intermedin.

The preceding experiment was repeated with 12 mice bearing 7-day-old melanoma. The intermedin injection was repeated every second day with a dose of 0.1 cc. for a period of 69 days.

---

* When intermedin is injected into the fish at a time other than the spawning period, the Elritze develops an intensive red coloration on the ventral skin. The testing of the tumor tissue extracts for intermedin was done by Miss G. Hildebrandt, to whom the author wishes to express his appreciation.

† An aqueous extract was prepared by Dr. H. R. Downes from the acetone-extracted dried anterior and middle lobes of the beef hypophysis according to the method of Zondek.[2] One cubic centimeter of this solution represents about 0.4 gm. of dried tissue. According to Zondek, the extracts prepared by his method, oxytocin and vasopressin are almost completely removed. However, we have not verified the above statement. The activity of the preparation was assayed according to the Zondek procedure.

[2] Zondek, B., and Krohn, H., *Klin. Woch.*, 1932, **11**, 405, 849, 1293.

The results of the experiment showed that the increased rate of tumor growth in the treated animals was practically the same as that of the preceding experiment. These results are shown in Fig. 1.

It may be stated that the stimulating action of intermedin upon the growth of the Passey mouse melanoma was no greater than that produced by the aqueous extracts of the pituitary.[1] In our earlier study extracts of both the anterior and middle lobes were used, whereas in this experiment only the extract of the middle lobe has been used. Therefore it is possible to state that the stimulating action is due solely to the hormone of the intermediary lobe.

Tumor metastases in the viscera of the treated and untreated animals did not occur but there was a marked phagocytosis of melanotic pigment in the liver, the spleen and the axillary lymph

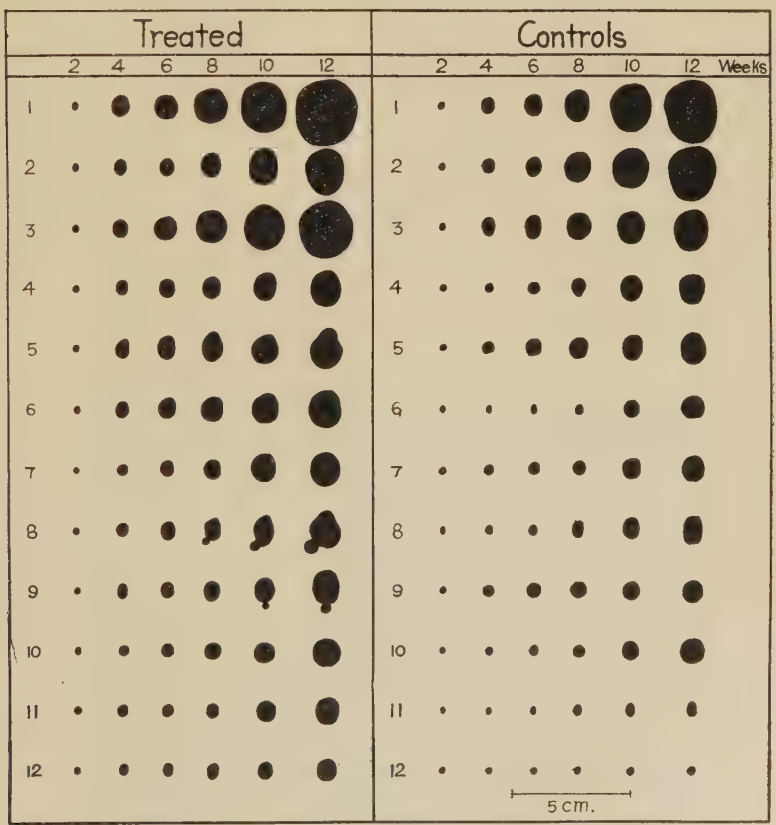


FIG. 1.

Showing stimulating effect of repeated injections of intermedin upon the growth of mouse melanoma.

nodes histologically examined when the tumors were approximately 180 days old. Very little melanotic pigment was found in the lungs and none in the kidney, heart and brain.

The author wishes to acknowledge his indebtedness to Dr. R. S. Ferguson for his suggestion of this problem.

7688 C

Effect of Divinyl Oxide on Isolated Intestinal Muscle.

S. A. PEOPLES* AND N. M. PHATAK. (Introduced by C. D. Leake.)

From the Pharmacological Laboratory of the University of California Medical School, San Francisco.

In the careful studies of Miller[1] on the effects of general anesthesia on the muscular activity of the gastro-enteric tract, it was shown that the relatively light anesthesia usually maintained with ethylene causes no marked changes in the tone or amplitude of contraction of intestinal muscles. On the other hand during the surgical stage of anesthesia with ether there is marked loss of tonus and almost complete inhibition of rhythmic and peristaltic contractions in stomach, small intestine, and colon. Divinyl oxide has been shown to have pharmacological properties resembling ethylene and ether, to which it is related chemically.[2] Its general physiological effects are less severe than those of ether, although it is a more powerful anesthetic agent. Since the action of divinyl oxide on intestinal movement has not yet been reported upon, it became of interest to determine what its relation might be to ether and ethylene in this regard.

Segments about 2 cm. long from the jejunum of a freshly killed rabbit were suspended by the Magnus method from a muscle lever, in oxygenated Locke's solution at 37.5° C. The drugs were added to the solution to saturation. Repeated trials were made with each drug in varying sequences of application on intestinal segments of 8 different rabbits.

Ether was always found to cause an immediate and marked loss of tone and inhibition of movement of such a muscle preparation

* Merck Fellow in Pharmacology.

[1] Miller, G. H., *J. Pharm. Exp. Therap.*, 1926, **27**, 41.

[2] Leake, C. D., Knoefel, P. K., and Guedel, A. E., *J. Pharm. Exp. Therap.*, 1933, **47**, 5.

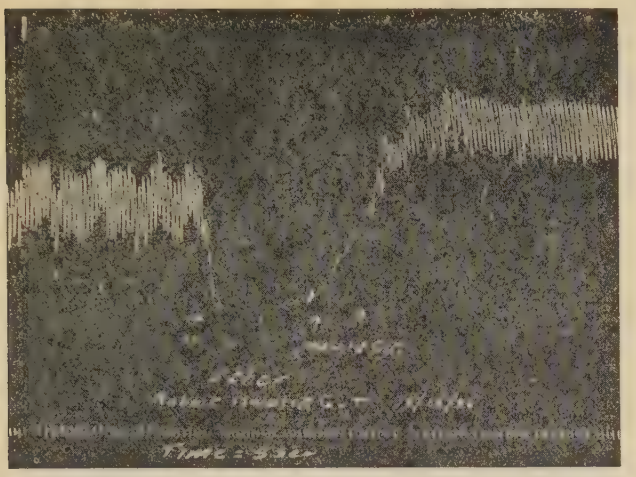


FIG. 1.
Effect of ether on isolated intestinal muscle.

(Fig. 1). Upon washing the ether out of the bath, normal tone and contractibility are promptly recovered. Ethylene bubbled to saturation in the bath was noted to cause a slight and transitory loss of the tone of the muscle, with occasional increase in amplitude of contraction (Fig. 2). Divinyl oxide added to saturation in the bath was observed uniformly to increase the tonicity of the intestinal segment. Sometimes this might be preceded by a slight loss of muscular tonus as in the case of ethylene (Fig. 3). If the muscle

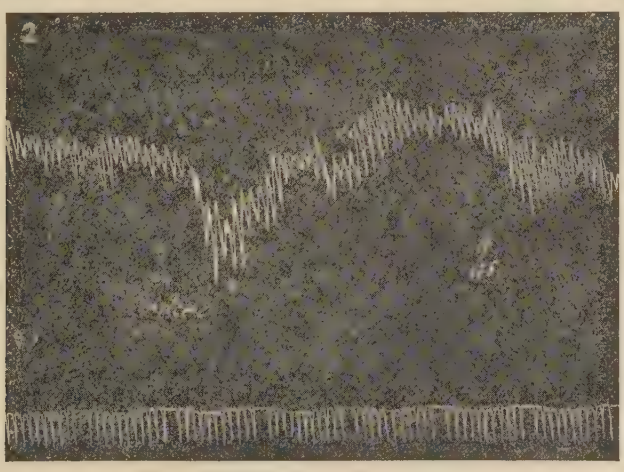


FIG. 2.
Effect of ethylene on isolated intestinal muscle.

FIG. 3.
Effect of divinyl oxide on isolated intestinal muscle.

tonicity is greatly augmented by the addition of divinyl oxide, the amplitude of contraction is usually reduced, and the increased tonicity is gradually lost as the oxygen bubbling in the bath removes the volatile agent.

Clinically ethylene anesthesia is felt to produce much less disturbing gastro-enteric symptoms than ether. The incidence of post-operative intestinal stasis and gas pain seems to be less after ethylene than after ether. This is in accord with the direct observation of Luckhardt and Lewis[3] that more active peristalsis is noted by surgeons during abdominal operations under ethylene than under ether. It is also in accord with Miller's findings,[1] and those reported here. Our observations on the action of divinyl oxide on isolated intestinal muscle indicate that in this respect as in other phases of its pharmacology, it has properties resembling ethylene more than ether. Indeed, since it definitely increases intestinal muscle tonus, it may be expected to be followed by even less post-operative intestinal stasis when used clinically than ethylene, which is reputedly so much better in this desideratum than ether.

[3] Luckhardt, A. B., and Lewis, D., *J. Am. Med. Assn.*, 1923, **81**, 1851.

7689 P

Tissue Culture Studies on Relation of Sarcoma to Leukosis of Chickens.*

J. FURTH AND E. L. STUBBS.

From the Department of Pathology, Cornell University Medical College, New York.

These experiments have been undertaken to determine whether sarcoma and leukosis can be produced by a single agent (virus).

Oberling and Guérin[1] described a virus that in their opinion produces leukosis, sarcoma and carcinoma. Troisier[2] confirmed their observations. Rothe-Meyer and Engelbreth Holm[3] and McIntosh[4] suggested that a single virus may produce both sarcoma and leukosis. During the first half of 1933 we isolated 2 transmissible strains that produce both sarcoma and leukosis and performed numerous experiments to determine whether each of these strains was caused by a mixture of 2 viruses or by a single virus. Some of these experiments are here described.

The strain used in this study (Strain 13, Stubbs and Furth) produces both sarcoma and leukosis. Sarcoma is readily grown *in vitro,* but leukotic blood cells perish within a few days in tissue cultures prepared in the usual manner. Experiments were undertaken to determine whether the ability of this strain to produce erythroleukosis would vanish with the disappearance of the primitive blood cells from the cultures, but it did not disappear. The cultures remained highly virulent after cultivation *in vitro* for 67 days and produced either sarcoma or sarcoma and erythroleukosis, as shown in Table 1.

Tissues of a sarcomatous tumor (Strain 13) were incubated *in vitro* in Carrel flasks in the usual manner, in clotted plasma containing approximately 0.5% of embryonic extract, with a supernatant liquid layer of chicken serum diluted with 3 times its volume of Tyrode solution and containing 0.5% of embryonic extract.

Although these sarcoma cells seldom digest the plasma clot, and the addition of foreign tissue is not required to support their growth, nevertheless they are highly virulent for chickens.

* This investigation has been supported by a Fund for the Study of Leukemia. Mr. Charles Breedis assisted in the work.

1 Oberling, Ch., and Guérin, M., *Bull. Assn. franç. étude cancer,* 1933, **22**, 180.

2 Troisier, J., *Bull. Assn. franç. étude cancer,* 1934, **23**, 225.

3 Rothe-Meyer, A., and Engelbreth Holm, J., *Acta path. Scand.,* 1933, **10**, 380.

4 McIntosh, J., *Brit. J. Exp. Path.,* 1933, **14**, 422.

TABLE I.
Result of Inoculation of Tissue Cultures of Sarcoma 13.

Age of tissue *in vitro*, days	No. of sub-passage	No. of fowls injected	No. of successful injections	Type of disease produced		Incubation period of tumor, days	Incubation period of blood changes, days	Length of life, days
				S*	S and E*			
12	I	4	4	2	2	15 to 25	25	25 to 77
23	III	5	4	2	2	14	28.5	28 to 37
35	III, IV	4	4	4	1	17	30	25 to 34
67	VIII to X	6	5	2	3	17 to 33	32 to 47	37 to 47

* S = sarcoma; E = erythroleukosis.

All chickens of the first 3 groups were Barred Rocks weighing approximately 350 gm.; the chickens of the last group were White Leghorns weighing 750 gm. The incubation periods shown are only approximate. "Length of life" means length of life after inoculation. Most chickens died and a few were killed *in extremis*. Two chickens of the last group are still alive.

The above data indicate that the virus of Strain 13 has multiplied *in vitro* and has retained during 67 days at 39° C. the ability to produce sarcoma or sarcoma and erythroleukosis. Erythroleukosis produced by this strain was associated with diffuse sarcomatosis of the bone marrow and spleen, and it might therefore be regarded as a secondary disturbance due to interference with erythrocyte formation.

The transmission experiments made with this strain are in conformity with this view. Microscopic studies of all instances of erythroleukosis produced by this strain revealed diffuse sarcomatosis of the blood-forming organs, with the exception of 2 cases in which the small sample of the marrow preserved for microscopic study showed only erythroleukosis. The assumption that the agents of sarcoma and leukosis are identical, the route of entry determining the type of disease,[1] is erroneous, for our leukosis Strains 1 and 2 do not produce Rous sarcoma even when introduced by intramuscular injection and the agent of sarcoma, Strain 13, here described produces extensive diffuse sarcomatosis of the spleen, bone marrow, and of several other organs, after intravenous inoculation.

The assumption of recent workers that a single agent may produce in some chickens pure leukosis and in others pure sarcoma or carcinoma requires stronger evidence than hitherto presented. Only one instance of carcinoma was observed in the study of our Strain 13, involving approximately 300 chickens, and all 3 chickens injected with tissues from this carcinoma remained healthy.

Alternative assumptions to the one just proposed are: (a) the

tissue cultures contain 2 viruses (a sarcoma and a leukosis virus) and both grow equally well in the presence of the sarcoma cells; (b) the culture contains one virus that stimulates to neoplastic growth both reticular and endothelial cells, as well as primitive erythroblasts.

Summary. A pure culture of chicken sarcoma is described (Strain 13) that elaborates a highly virulent virus or viruses capable of producing both sarcoma and erythroleukosis.

7690 C

Blood Amylase Response to Acetylcholine and its Modification by Physostigmine and Atropine.

WILLIAM ANTOPOL, ARTHUR SCHIFRIN AND LESTER TUCHMAN.
(Introduced by Harry Sobotka.)

From the Laboratories of the Mount Sinai Hospital, New York City, and the Bayonne Hospital and Dispensary, Bayonne, New Jersey.

In the course of some experiments on glycogen deposition, it occurred to us that the vagus, which is thought to influence the deposition of glycogen in tissue,[1] might do this through action on the blood amylase. With this in mind, we have studied, in dogs, the variations in blood amylase titre after the intramuscular administration of acetylcholine,* the so-called vagus substance. For the determinations, we have used the viscosimetric method of Elman,[2] employing the same stock solution of starch for all the determinations in each individual experiment. Determinations were done at intervals varying from 15 minutes to 24 hours. Marked increases in blood amylase values, at times more than 4 fold, were found in 8 experiments on 7 dogs. Table I illustrates these findings.

The fasting blood amylase titre varied between 24 and 60 units, which is in accord with the values found in the literature. For control, determinations were performed on 13 fasting dogs at intervals varying from 15 to 24 hours. It was found that on the same day, using the same stock solution of starch, the variations in the individual dogs did not exceed 3 units. Further, the adminis-

[1] Debois, G., *Compt. rend. Soc. biol.*, 1930, **103**, 546.

* The beta methyl derivative of acetylcholine (Mecholin-Merck) was used throughout these experiments.

[2] Elman, R., Arneson, N., and Graham, E. A., *Arch. Surg.*, 1929, **19**, 943.

TABLE I.
Influence of Acetylcholine on Blood Amylase in Dogs.

Dog	Wt. kg.	Total dosage of acetylcholine mg.	Blood Amylase Units After					
			Fasting	¼	1	3	6	24 hr.
L	30	20	54	98	240			105
L	30	25	32	141	143		139	
38	30	20	46		67	71		51
33	19	10	50			178	167	
I	11	10	28		160			
II	14	10	60		184			
III	12	10	28		63			
15	13	10	29		67			

tration of saline, 0.5 mg. of epinephrine, or 3 mg. of histamine did not cause a significant change from the fasting blood amylase level.

Loewi and his coworkers[3] have demonstrated that physostigmine inhibits the physiological destruction of acetylcholine. Hence, the action of physostigmine, given alone and in conjunction with acetylcholine, was next studied in order to determine its influence on the blood amylase response to acetylcholine administration in dogs. When physostigmine alone was injected intramuscularly, there was no appreciable change in the blood amylase titre. However, with the administration of acetylcholine after physostigmine, the rise in blood amylase was far in excess of that obtained with a similar dose of acetylcholine alone. This was found in all of 7 experiments on 4 dogs. (In one case it was over 13 times the fasting blood amylase

TABLE II.
Influence of Administration of Physostigmine 1 Hour Before Acetylcholine.

Dog	Wt. kg.	Fasting amylase units	Dose of physostigmine mg.	Blood amylase units 1 hr. later	Dose of acetylcholine mg.	Blood amylase units after			
						3	4	6	24 hr.
L	30	42	3.5	38	0	41			
L	30	38	3.0	28	10	244		276	86
L	30	48	6.0	54	5	555	680		128
15	12	25	3.0	35	10	114	109		
90	12	26	3.0	75	10	200			

Simultaneous Administration of Physostigmine and Acetylcholine

Dog	Wt. kg.	Blood amylase units fasting	Dose of physostigmine. mg.	Dose of acetylcholine mg.	Blood amylase units after		
					1	3	24 hr.
L	30	44	3.5	20	171	525	79
30	14	38	0.85	5	96	222	57

[3] Engelhardt, E., and Loewi, O., *Arch. exp. Path. Pharmak.*, 1930, **1**, 150.

level.) In this group of experiments, smaller doses of acetylcholine were used than in Group I. This was necessary because the original dosage of acetylcholine when combined with physostigmine frequently proved lethal. In spite of this reduction in dosage, the blood amylase reached even higher levels than in Group I. Table II illustrates these findings. (Note that dog L in Table II is the same dog as L in Table I.)

Since atropine is known to abolish the vagus response, we next studied the effect of atropine injection preceding acetylcholine administration in dogs. When atropine alone was injected intramuscularly, there was no appreciable change in the blood amylase titre. The subsequent administration of acetylcholine in effective doses failed to produce appreciable change in the blood amylase. Table III summarizes these results on 3 dogs.

TABLE III.
Blood Amylase Response to Acetylcholine After Administration of Atropine.

Dog	Wt. kg.	Fasting blood amylase units	Dose of atropine mg.	Blood amylase units just prior to injection of acetyl-choline	Interval between atropine and acetyl-choline min.	Dose of acetyl-choline mg.	Blood amylase units 3 hr.
L	30	49	2	52	15	20	47
I	11	25	1.2	24	45	10	26
III	12	24	1.2	22	45	10	26

Note: These dogs are the same as dogs L, I, and III of Table I.

Summary. 1. Intramuscular administration of acetylcholine in dogs results in an increase in the blood amylase titre. 2. Physostigmine markedly increases the blood amylase response to acetylcholine. 3. Previous atropine administration inhibits the blood amylase response to acetylcholine.

7691 P

Specific Soluble Substance of Pneumococcus in Lungs of Dogs Recovered from Experimental Lobar Pneumonia.

JAMES B. GRAESER.

From the Department of Medicine and the Douglas Smith Foundation for Medical Research, University of Chicago.

That the soluble specific substance of the pneumococcus may remain in tissues infected with this organism even after the inflammatory exudate has disappeared was first suggested by the studies of Dochez and Avery[1] on the excretion of "S" substance in the urine of cases of lobar pneumonia in human beings. They found that the soluble specific substance was excreted in some cases for days or weeks after recovery from the disease. In some instances this continued excretion of "S" could be correlated with delayed resolution, but in other cases this was not the explanation. It would seem probable that in these latter instances "S" remained stored in the pulmonary tissues for some time after the other constituents of the inflammatory exudate had apparently disappeared.

A direct approach to this problem cannot be made in human beings because of obvious difficulties. However, the availability of an experimental animal in which a pneumococcus pneumonia may be induced makes possible the determination of "S" substance in the lungs at various intervals after recovery. The present report is a record of preliminary studies of the persistence of "S" substance in the lungs of dogs recovered from experimental lobar pneumonia.

Healthy adult male dogs were infected with Type I pneumococci by the method of Terrell, Robertson, and Coggeshall.[2] Some of these dogs had been previously "vaccinated" by spraying formalized Type I pneumococci into the bronchial tree. Two dogs in the group had been similarly treated with a 0.9% salt solution as controls. The "vaccination" procedure had been carried out in the course of a separate experiment but the dogs after recovery were utilized for this study. The other animals in the series had received no preliminary treatment.

After inoculation the dogs were examined regularly with the fluoroscope to determine the progress and extent of the pulmonary lesion and to ascertain as accurately as possible by this means the

[1] Dochez, A. R., and Avery, O. T., *J. Exp. Med.*, 1917, **26**, 477.

[2] Terrell, E. E., Robertson, O. H., and Coggeshall, L. T., *J. Clin. Invest.*, 1933, **12**, 393.

TABLE I

Dog No.	Condition of dog before infection	Time of killing animals: Days after inception of infection	Time of killing animals: Days after disappearance of lesion	Highest dilution of tissue extract giving precipitin reaction: Infected Lung	Highest dilution of tissue extract giving precipitin reaction: Uninfected Lung	"S" substance in blood serum and Urine: Serum	"S" substance in blood serum and Urine: Urine	Histological appearance of infected lung
17G	Normal	2		1-320	0	+	+	Uniform exudate of polymorphonuclear cells
32H	"	4		1-384	0	+	+	Uniform exudate of polymorphonuclear cells
34H	"	6		1-384	0	0	+	Late stage resolution. Exudate principally macrophages
25F	"	12	8	1-512	0	0	0	Thickened alveolar walls
6F	Vaccinated	13	7	1-384	0	—*	—	Still slight macrophage reaction
5F	"	13	7	1-384	0	—	—	Most areas normal. A few alveoli contain detached monqnuclear cells
23F	Saline "Vaccinated"	18	10	1-480	0	0	0	Alveolar walls in some areas still thickened and some of the septal cells are swollen
18F	Vaccinated	25	15	1-240	0	0	0	Alveolar walls still slightly swollen. No exudate
14F	Saline "Vaccinated"	57	50	1-128	0	0	0	Normal
28F	Normal	70	60	1-256	0	0	0	"
20F	Vaccinated	109	98	0	0	0	0	"
17F	"	109	96	1-128	0	0	0	"

*— = Determinations not made.

time of completion of resolution. At different intervals after inoculation the animals were sacrificed and the previously infected and normal lobes were removed for study. Histological sections were prepared for microscopic examination.

For the demonstration of "S" substance the lung tissues were frozen in liquid air and crushed to a fine powder in an apparatus previously described.[3] Two volumes of 0.9% salt solution were added to each gram of tissue. The brei was then placed in the ice box for extraction. Twenty-four to 48 hours later the material was centrifuged and the supernatant fluid filtered through a small Seitz pressure filter. Type I antipneumococcus serum was then overlaid with the clear filtrate in varying dilutions, and the highest dilution showing a precipitin reaction determined. It is to be noted that the lowest dilution tested thus was 1-3.

A purely qualitative determination for the presence of "S" in the blood serum was made by the method described by Amoss.[4] Urine removed from the animal at the time of death was tested for "S" by simply overlaying a small amount over antiserum.

The data obtained are summarized in Table I. It should be kept in mind that the pneumonic lesion in dogs passes through the stages of development and regression more rapidly than in human beings. Furthermore, there occurs the same variation in rate of these changes in individual dogs as one sees in a series of cases of the human disease. Thus clearing of the lesion as determined by fluoroscopy may be complete in 4 days, as in dog 25 F, or may not take place for 13 days, as in dog 17 F. Fluoroscopic examination, of course, is not adequate to demonstrate actual restitution to normal, histologically, but is sufficiently accurate for the comparative purposes of this study.

The data obtained thus demonstrate that "S" substance may be present in detectable amounts in previously infected lobes 2 to 3 months after the inflammatory exudate has disappeared. The question may, of course, be raised as to the rôle vaccination may have played in causing retention of "S" substance in some of these cases. Experiments on normal animals are now in progress to check this point. The lung of a normal animal, dog 28 F, however, contained "S" substance 2 months after resolution had taken place.

Soluble substance was not found in the uninfected lobe at any stage of the disease where the lowest dilution tested was 1-3. In

[3] Graeser, J. B., Ginsberg, J. E., and Friedemann, T. E., *J. Biol. Chem.*, 1934, **104**, 149.

[4] Amoss, H. L., Proc. Soc. Exp. Biol. and Med., 1930, **28**, 23.

several cases, not included in this series, the tissue extract from the uninfected lobes has been concentrated to equal an undiluted tissue extract and "S" substance was not demonstrable.

7692 C

Hemoglobin Studies. I. In Rachitic Chickens: Effect of Ultraviolet Irradiations.*

GEORGE H. MAUGHAN.

From the Department of Physiology, Cornell University.

A study of the effect of ultraviolet irradiation on the amount of hemoglobin of the blood was made on 3 pens of White Leghorn chickens (10 chickens each pen) grown in complete absence of sunshine. The room, 20′ x 30′, received moderate lighting from two 60 watt Mazda lamps. Small brooders supplied with 60 watt carbon filament lamps furnished the brooder heat for individual pens.

Pen 1, the normal controls, received 2 minutes daily irradiation from a quartz mercury arc lamp at 30 inches distance. Pen 2 had no ultraviolet irradiation. Pen 3 received no ultraviolet until after hemoglobin studies on rachitic chickens were made.

The irradiated chickens developed smooth plumage, yellow shanks and bills, and the male birds large red combs. (These organs do not develop until later in the females.) All of these chickens showed every sign of normal growth.

The non-irradiated chickens were less than 2/3 the size of the controls (Table I). Many showed extreme weakness and deformed

TABLE I.
Comparative Size, Hemoglobin and Blood Calcium of Normal Irradiated (Control), Non-irradiated and Rachitic Irradiated (Healing) Chickens.

Pen	Wt. in gm.	Hb 14.5 = 100%	Blood Ca mg. per 100 cc.	Hb later	
				10 days	25 days
I	315	10.3	12.6	10.3	10.9
II	197	8.41	6.73	8.00	dead
III	203	6.52		8.41	11.6

joints and bones due to rickets. The feathers were rough and the shanks and bills were light, almost white, in color.

At 7 weeks of age hemoglobin studies were made. These aver-

* Aided by a grant from the Heckscher Research Foundation.

age readings were for the 3 pens, 10.3; 8.41; and 6.52 gm. Hb per 100 cc. blood, respectively. (Determinations were made by the Hellige Solid Plane Hemometer reduced to the standard 14.5 gm. Hb per 100 cc. of blood.)

After these tests were made Pen 3 received daily irradiations (10 minutes each day) from the quartz lamp. At the end of 10 days the comparative Hb readings were 10.3; 8.00; and 8.4. It will be noted that the irradiated rachitic chickens (Pen 3) had already increased their Hb nearly 2 gm. per 100 cc. of blood in 10 days. Two weeks later the chickens in Pen 2 were all dead, due to rickets, while Pens 1 and 3 had the following Hb tests: 10.92 and 11.6 respectively.

Studies made by Cook and Harmon,[1] Dukes and Schwarte,[2] and Hart, Elvehjem, Kemmerer and Halpin[3] show that the hen has a lower hemoglobin content than human beings. Our own studies[4] made on chickens of all ages confirm their results and give figures ranging between 50 and 80% of the average for man.

Rachitic chickens, as might be expected, have a low hemoglobin. The amounts as compared to normal chickens range between 43 and 85%. In a number of tests on very rachitic birds the hemoglobin was as low as 5 gm. per 100 cc. of blood.

Ten days' irradiation caused a definite rise in hemoglobin content in Pen 3, and at the end of 3 weeks it was higher than in the controls. One might interpret this to be due to the indirect effects brought about because of increased physiological activity very evident in many systems, especially the endocrines and the blood-forming cells of the bone marrow.

---

[1] Cook, S. F., and Harmon, I. W., *Am. J. Phys.*, 1933, **105**, 407.

[2] Dukes, H. H., and Schwarte, L. H., *Am. J. Phys.*, 1931, **96**, 89.

[3] Hart, E. B., Elvehjem, C. A., Kemmerer, A. R., and Halpin, J. H., *Poultry Science*, 1929, **9**, 92.

[4] Not published.

7693 P

Immunity to Cross-Infection in Avian Malaria Due to Plasmodium Vaughani.

REGINALD D. MANWELL. (Introduced by Walter S. Root.)

From the Department of Zoology, Syracuse University.

For many years it was believed that infection with one species of avian malaria conferred protection against subsequent infection with that species only, and one of the tests by which a given species could be differentiated from another was to inoculate a subpatent or chronic infection of the former with the suspected new species. Recently it has been shown by Gingrich[1] and others that this is not always the case, but this method is still an important test of specificity.

The species used in the present work was originally described by Novy and MacNeal[2] from the common robin in 1904, and the strain employed was isolated from a catbird caught in Syracuse in April, 1934. The other species with which *P. vaughani* was crossed were secured from sources previously mentioned by the author.[3, 4] Female canaries were used throughout.

Before inoculating a bird with a latent infection the continued presence of an infection was checked either by microscopic examination or (since this is usually negative) by subinoculation into fresh birds. Inoculations were generally intravenous, and large doses of parasites were usually used. Ten series of 3 birds each (in addition to one or more controls in each series) were used, 5 being chronic cases of *P. vaughani* and the others consisting of one series each of *P. cathemerium, praecox (relictum), circumflexum, elongatum,* and *rouxi.* Each series of chronic *vaughani* infections was inoculated with one of the other species named, and the experiment was then reversed, using chronic infections of the latter.

The results show that a subpatent (chronic) infection with *P. vaughani* confers little if any protection against subsequent infection with the 5 other species named, and the reverse of this relationship appears to hold true also, except that there is some indication that a pre-existing infection with *P. praecox (relictum)* gives a partial immunity to subsequent infection with *P. vaughani.* That

1 Gingrich, Wendell, *J. Prev. Med.*, 1932, **6**, 197.

2 Novy, F. G., and MacNeal, W. J., *Am. Med.*, 1904, **8**, 932.

3 Manwell, R. D., *Am. J. Hyg.*, in press.

4 Manwell, R. D., *Am. J. Trop. Med.*, in press.

neither *P. rouxi* nor *P. vaughani* confers any protection against the other is rather difficult to demonstrate with certainty because of the very close morphological resemblance between these 2 species, but all the evidence seems to point that way. Birds having a chronic infection of *vaughani*, for example, usually show no parasites after a few weeks, whereas those infected with *rouxi* generally show parasites for months or years. Chronic cases of one of these 2 species, when infected with the other, behave as do pure chronic infections of *rouxi*. In view of the close resemblance of these 2 species, the absence of cross-immunity is surprising. It confirms the evidence offered by the differences in natural hosts, in geographical distribution, in morphology, and in type of infection, that these 2 very similar species are in reality distinct.

An incidental result of this study has been the finding of 2 cases of complete recovery from *vaughani* infection, as shown by failure to infect fresh birds with massive doses of the blood of these cases. This is a very rare occurrence in avian malaria of any kind. Superinfection was not tried in these cases for unavoidable reasons, but it is easily possible in birds cured by drugs, and is impossible in cases of chronic infection.

7694 P

Observations on a Dog Maintained for Five Weeks Without Adrenals or Pancreas.

C. N. H. LONG AND F. D. W. LUKENS.

From the George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia.

We have previously reported[1] some preliminary observations on totally adrenalectomized-depancreatized cats. Several workers in the past have attempted this double operation upon dogs and in no instance have the animals survived more than a few hours. Such results as have been obtained have led to the conclusion that total adrenalectomy had no influence upon the subsequent course of the diabetes. During the past year we have completed the double operation upon a dog which was observed over a period of 5 weeks.

The dog (a male weighing 11.2 kilos) was totally depancreatized on March 9th, 1934, the left adrenal removed on March 20th, 1934,

[1] Long, C. N. H., and Lukens, F. D. W., *Science*, 1934, **79**, 569.

and the right on May 4th, 1934. The animal lived until June 8th, 1934.

Following the pancreatectomy the animal was easily stabilized on a diet of 300 gm. liver, 50 gm. raw pancreas and 20 gm. sugar daily with 20 units of insulin administered in 2 doses. On this regime the urinary glucose averaged about 4 gm. per day. The fasting overnight blood sugar ranged from 191-254 mg. %. During this period of 10 days the weight decreased to 10.3 kilos.

After the unilateral adrenalectomy the daily glycosuria still averaged 4 gm. on the same diet and insulin, while the blood sugar ranged around 270 mg. %. Reduction of the insulin to 10 units daily keeping the diet the same resulted in an increase of the urinary glucose to 16 gm. daily. The animal was then restored to the previous regime and maintained on it in good health for a further 6 weeks. During this time no alterations occurred in the character of the diabetes while the weight at the end of the period was 9.1 kilos.

Following the second adrenalectomy the injection of a preparation of cortical extract, kindly supplied by Parke, Davis and Company, was commenced. Since this was not a very potent preparation 10 cc. daily were injected intraperitoneally, and in addition about 4 gm. of NaCl were included with the food. The animal was brought into balance again by diet and insulin, as we wished to determine the insulin requirement on diets similar to those used when some adrenal tissue was present. The duration of life after removal of the remaining adrenal was 35 days.

Four days after operation the animal was placed on a diet of 300 gm. of liver, 50 gm. of raw pancreas and 40 gm. of sucrose. Four units of insulin daily now kept the glycosuria at about 2 gm. per day. The fasting overnight blood sugars ranged from 44-160 mg. %.

After 5 days of this regime the diet was increased to 400 gm. of liver, 50 gm. of pancreas and 60 gm. of sucrose with the same insulin dosage (4 units). The glycosuria only increased to 6 gm. daily and the fasting blood sugars for a 3-day period were 68, 101 and 132 mg. %. For one day on this diet insulin was withdrawn and the urinary glucose promptly rose to 63 gm. The sucrose in the diet was then omitted and the animal kept on meat alone without insulin for a further 3 days. The glycosuria was 20 gm. daily and the fasting blood sugars were 143, 270 and 257 mg. %. The D/N ratio on the third and fourth days of insulin withdrawal were 1.6 and 1.7 respectively. The animal was then returned to the diet of

400 gm. of liver, 50 gm. of pancreas, 60 gm. of sucrose and 4 units of insulin daily for a further 17 days, after which time it collapsed and died 2 days later. At autopsy a subdiaphragmatic abscess was found, associated with a terminal peritonitis. Prior to this development the animal had been well and lively, and at the time of death it weighed 8.1 kilos. No adrenal or pancreatic tissue was found at autopsy.

In this animal the removal of one adrenal had apparently no effect on pancreatic diabetes. This conclusion is reached since after the unilateral adrenalectomy the same amount of insulin was required with the same diet to keep the glycosuria at the same level.

After removal of all adrenal tissue the most striking feature was the ability of small amounts of insulin, one-quarter to one-fifth the original amount, to keep the glycosuria at a very low level, in spite of feeding of as much as 60 gm. of sucrose daily in addition to 450 gm. of meat. The available carbohydrate of this diet was about 127 gm. yet only 4 units of insulin were required to keep the urine almost sugar free.

On the other hand it is quite apparent from a study of the periods when insulin is withheld that the animal did not possess a normal capacity to metabolize carbohydrate. How long such an animal would have survived if not treated with insulin remains to be determined.

7695 P

Indirect Method for Determining Blood Pressure in Small Animals.

J. Q. GRIFFITH, JR.* (Introduced by A. N. Richards.)

From the Robinette Foundation, the Medical Clinic, and the Pepper Laboratory of the Hospital of the University of Pennsylvania.

Experimental problems requiring the repeated taking of blood pressure have, in the past, required the use of fairly large animals. Various indirect methods suggested for small animals have, as a rule, proven unsatisfactory. Griffith and Collins[1] reported an indirect method for obtaining blood pressure in man by capillary observation with simultaneous arterial compression. This method has now been modified for use in small animals.

* Instructor in Medicine and Heckscher Fellow in Medical Research.

[1] Griffith, J. Q., Jr., and Collins, L. H., Jr., *Am. Heart J.*, 1933, **8**, 671.

White rats under nembutal anesthesia were used. The dorsum of one foot was depilated, either with barium sulphide or, better, with a razor. A special blood pressure cuff was then wrapped about the thigh and fastened with adhesive. This cuff is 2.7 cm. wide and encloses a circular pressure bag 2.4 cm. in diameter. This cuff conforms in design to a portion of a ring enclosed between 2 concentric circles, the outer circle having a radius of 5 cm. and the inner a radius of 2.3 cm. The 'ring' or cuff is thus seen to be 2.7 cm. wide, and any convenient length may be used, 25 cm. measured along the greater circle being quite satisfactory. The pressure bag is inflated from a rubber bulb which is compressed by a thumb screw, the whole system being connected through a T-tube to a mercury manometer. This type of cuff shows little tendency to slip, but if it does a stitch or two can be taken through the skin.

The foot is now gently supported by a mould of plasticine, and a drop of immersion oil placed on the shaven area. A strong light, cooled by passing through water, is focused on this area, and it is observed under the low power objective of the microscope.

Ordinarily in such a preparation the field will be full of vessels, many of which will show distinct flow. For the purpose of this communication we will make no attempt to differentiate between arterioles, venules, and capillaries, but simply refer to them all as minute vessels. Roughly, there are 2 general types of vessel visible: (1) very red, very distinct vessels with relatively wide channels, rarely showing distinct segmentations in their homogeneous stream, and either flowing very slowly or not flowing at all; (2) relatively thin and faint vessels in which the flow is very fast and the stream invariably granular in appearance. Readings using the first type of vessel are inaccurate. A vessel of the second type is therefore selected and the pressure in the cuff is slowly raised until all flow stops. After cessation of flow is complete the pressure is lowered 3-5 mm. at a time and the level where distinct flow begins is recorded. This end point in a favorable case is extremely sharp. Usually 3-4 such vessels can be observed in a single field, and it is surprising how nearly identical the time of onset of flow is in each. This reading should be repeated several times, using other vessels, and, in readings made a few minutes apart, there will usually be agreement within 5 mm. (provided the anesthesia is constant). Theoretically, the point of cessation of flow as the pressure is raised and of return of flow as it is lowered are identical. Actually, the point of return is by 3-5 mm. the lower.

Blood pressure readings were obtained by this method in over a

hundred rats. The figures obtained varied between 140 and 60, with 9/10 falling between 80-100 mm. It was then thought advisable to compare readings obtained by this method with those secured by direct needling of an artery. Both readings were made in the same 10 minutes, in an animal which remained in apparently the same stage of anesthesia, and in which the direct reading was obtained virtually without blood loss.

TABLE I.
Blood Pressure in Millimeters of Mercury.

Animal	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Indirect†	68	97	85	91	112	74	96	105	75	65	102	88	110	70	88
Direct‡	74	91	76	94	102	78	88	112	81	77	98	80	112	75	94

† Using left femoral artery.
‡ 1-5 inclusive using abdominal aorta, 6-15 inclusive the left carotid.

7696 P

Observations with Oerskov's Milk Bacillus.

L. DIENES.

From the Department of Pathology and Bacteriology, Massachusetts General Hospital.

Oerskov[1] reported that in the cultures of certain *fluorescens* strains isolated from milk, if the media contains saccharose, masses of tiny non-stainable granules develop which multiply independently from the bacteria. The cultures on ordinary agar plates correspond in every respect to the cultures of other *fluorescens* strains; on saccharose agar plates the colonies consist mostly of an amorphous substance in which strands of bacteria are embedded. By direct examination under the microscope or by the usual bacterial staining methods in the amorphous substance, no form elements are visible. In dark background preparations it seems to consist of innumerable tiny granules small enough to pass a Berkefeld filter. If the development of the colonies is examined under the microscope, it is seen that the bacilli and the amorphous substance start to grow separately. The amorphous substance grows in tiny round transparent colonies with a more refractile zone at the center. Most of these colonies coalesce later with the colonies of the bacillus and those which remain free grow only to a size of 0.1-0.2 mm. Oers-

[1] Oerskov, T., *Zentrbl. f. Bakt. I. O.*, 1931, **120**, 310.

kov did not succeed in growing the granules separately from the bacteria but when a bacillus emulsion was killed by moderate heat and planted, the granule colonies grew between the dead bacilli. The conclusion of Oerskov is that a virus-like organism is growing in symbiosis with the bacillus.

We succeeded at the first attempt in isolating Oerskov's bacillus and were able to verify his most important observations. In the following points our observations supplement those of Oerskov or are at variance with them.

The granules can be cultivated separately from the bacillus. The granule colonies are of 2 distinct types; one is smaller, perfectly transparent and gives no growth in transplants; the other is somewhat larger and, as already indicated by Oerskov, it contains a more refractile central zone. This central zone is really grown into the agar. If the plates are washed, they remain unchanged; if the agar is stained, they are visible as small unstained spots in the agar in which no bacteria are visible. The larger colony type can usually be transplanted and in the transplants numerous colonies of the first type grow which then cannot be further transplanted. If a fresh saccharose broth culture is filtered through a Berkefeld filter and planted immediately after filtration, innumerable tiny colonies belonging to the first type develop in the transplants. Bacteria are never reproduced in the filtrates or in the transplants from granule colonies.

The bacteria exert no growth promoting effect on the granules and there is no reason to assume a symbiotic relationship between them. The growth of the transplants is not affected by the presence of dead or living bacteria in the agar or on the agar surface or by the proximity of bacterial colonies.

The granules originate from disintegrating bacilli. The most appropriate medium for the study of the derivation of the granules from the bacteria is dextrose broth. In the cultures grown in this medium granules are not visible in dark background preparations but a few minutes after addition of saccharose a large number of the bacilli disintegrate and are transformed into clumps of the characteristic granules. We observed a Gram negative bacillus, not belonging to the *fluorescens* group, which showed the same phenomena on saccharose agar as Oerskov's bacillus. The bacilli in the culture of this strain grew to very large crescent shaped forms before they disintegrated. The granules continue to multiply after the disintegration of the bacillus. In most cultures, especially in dextrose and saccharose broth, there are also invisible elements

which give rise to granule colonies. If the broth is plated on saccharose agar, direct examination under the microscope shows that granule colonies grow mostly in places where no bacteria were deposited.

The phenomena observed with Oerskov's bacillus show, in addition to characteristic differences, close similarities to the phenomena described in previous notes with Gram-positive aerobic bacilli.[2, 3] As we have already indicated, these phenomena are probably closely related to the mucoid fermentations and the production of bacterial capsules.

We have formed so far no definite opinion concerning the nature of the amorphous substance and its connection with the bacteria, though it is probable from analogies to the Gram positive bacteria, that it contains living elements different from the usual bacterial forms.

7697 P

Presence of Vitamin B_1 in the Gastric Juice.

S. A. KOMAROV. (Introduced by B. P. Babkin.)

From the Department of Physiology, McGill University, Montreal.

Impaired functioning of the digestive system was for a long time known to be one of the earliest and most prominent manifestations of experimental vitamin-B deficiency. A diminution of gastric secretion was also reported repeatedly (Miyadera,[1] Shinoda,[2] Farnum,[3] Gilman[4]). The data concerning the gastric secretion were however not very consistent, and furthermore there were considerable difficulties in interpretation for the reason that the experiments quoted above were complicated by many other factors, particularly by a loss of appetite and usually a high degree of inanition in the experimental animals. Recently it was demonstrated in this laboratory that a marked diminution of gastric secretion occurs in dogs on

[2] Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1205; 1934, **31**, 1211.

[3] Peirson, O., and Dienes, L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1208.

[1] Miyadera, K., *Biochem. Z.*, 1921, **124**, 244.

[2] Shinoda, G., *Z. f. d. ges. exp. Med.*, 1924, **40**, 274.

[3] Farnum, M. B., *Arch. Int. Med.*, 1926, **37**, 42.

[4] Gilman, A., Dissertation, Yale University, 1931. Quoted from Cowgill, G. R., *J. Am. Med. Assn.*, June 18, 1932.

a diet lacking vitamin B even under conditions where the animals preserve an excellent appetite and well maintain their body weight (Babkin,[5] Webster and Armour[6, 7]). The withdrawal of yeast, as a source of vitamin B, from the standard diet practically abolished the gastric secretion in response to sham-feeding, to histamine, and to intra-intestinal administration of alcohol, as well as to introduction of food into the duodenum. The importance for normal gastric secretion of an adequate supply of vitamin B_1 was emphasized recently by Cowgill and Gilman.[8] The fact that with a diet lacking vitamin B the gastric glands are resistant to *any* kind of stimuli suggests that some of the factors concerned in vitamin B complex may play a vital part in the intimate mechanism of gastric glandular activity. In such a case one might expect the presence of such a factor also in the product of activity, *i. e.*, in the gastric secretion.

The experimental study which we have carried out along these lines has indeed provided ample evidence that at least the anti-neuritic principle, vitamin B_1, is a normal constituent of canine gastric juice.

The presence of an anti-neuritic factor in pure normal gastric juice of the dog was demonstrated by means of curative tests on polyneuritic pigeons (technique according to Kinnersley, Peters and Reader,[9] Guha and Drummond[10]). Gastric juice was collected on sham-feeding from 3 dogs with gastric fistula and oesophagotomy, which were receiving a diet of fresh raw meat, milk and oatmeal porridge. Only water-clear secretion was used and all the necessary measures were taken to prevent any growth of micro-organisms in the gastric juice or in the concentrates prepared from it. The anti-neuritic activity was demonstrated in the concentrates, which were prepared from the protein-free filtrate of gastric juice by adsorption on Fuller's earth at pH 4.5, followed by extraction of the adsorbates with $Ba(OH)_2$. Curative day doses for pigeons as determined with the above-mentioned concentrates were equivalent to from 100 to 200 cc. gastric juice, containing respectively 0.8 to 1.6 mg. nitrogen and 7 to 14 mg. organic material. In an attempt to investigate the chemical properties of the anti-neuritic constituent

[5] Babkin, B. P., *Can. Med. Assn. J.*, 1933, **29**, 5.

[6] Webster, D. R., and Armour, J. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1297.

[7] Webster, D. R., and Armour, J. C., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 463.

[8] Cowgill, G. R., and Gilman, A., *Arch. Int. Med.*, 1934, **53**, 58.

[9] Kinnersley, H. W., Peters, R. A., and Reader, V., *Biochem. J.*, 1928, **22**, 276.

[10] Guha, B. C., and Drummond, J. C., *Biochem. J.*, 1929, **23**, 880.

of gastric juice it was found that this substance belongs to the group of nitrogenous bases not precipitable by lead acetate in acid, neutral or slightly alkaline medium, but precipitable by an excess of phosphotungstic acid in acid medium (5% sulphuric acid). On fractionation by the silver-baryta method all the anti-neuritic activity was recovered in the silver precipitate obtained at pH between 5.5 and 7.0. The activity of the concentrate prepared from this silver fraction by extraction with a slight excess of hydrochloric acid corresponded to 75 to 200 cc. of gastric juice as curative day doses for pigeons. This amount of the fraction contained from 0.15 to 0.4 mg. nitrogen. The chemical properties and the mode of action of the concentrates studied strongly suggest that the active principle is vitamin B_1. Therefore vitamin B_1 should be regarded as a normal constituent of canine gastric juice.

7698 P

Haemodynamic Effects of Extracts from Traumatized Limbs.

N. W. ROOME AND HARWELL WILSON. (Introduced by D. B. Phemister.)

From the Department of Surgery, University of Chicago.

Two theories have recently received most attention as to the etiology of traumatic shock. These are (1) the absorption of metabolic toxins from the traumatized area (Cannon[1]) and (2) the local loss of blood and/or plasma (Blalock,[2] Phemister[3]) The work of various investigators showing certain tissue extracts to be circulatory depressants has frequently been cited in favor of the "toxic theory". The fact has been largely overlooked, however, that one may obtain both pressor and depressor substances from most tissues, and that either type may predominate according to the method of extraction (Collip[4]).

The present experiments are upon the effects on the blood pressure of extracts from traumatized limbs obtained by an hydraulic press. This method of extraction probably does not alter the proportions of the various hemodynamic substances present in the tissue.

[1] Cannon, W. B., Traumatic Shock, 1923.

[2] Blalock, A., *Arch. Surg.*, 1930, **20**, 959.

[3] Parsons, E., and Phemister, D. B., *Surg. Gynecol. and Obst.*, 1930, **51**, 196. Roome, N. W., Keith, W. S., and Phemister, D. B., *Ibid.*, 1933, **56**, 161.

[4] Collip, J. B., *J. Physiol.*, 1928, **66**, 416.

Twelve experiments were performed on dogs as follows: One animal was anesthetized, and one hind leg traumatized repeatedly by means of a padded hammer until the arterial blood pressure remained at a low level, usually 60 to 86 mm. Hg. The periods of traumatization (2-3 minutes) were interspersed with 10 minute periods for observation of the arterial blood pressure level. In no case did the traumatization break bones or rupture the skin. This leg was then amputated, skinned, and the muscles minced and extracted in an hydraulic press at a pressure of 375 kg./cm^2. A considerable amount of bloody fluid ran off during the dissection of the leg; this was given to the animal along with the muscle extract in 5 cases, and separately in 7 cases. In all cases the extract was administered to a second animal by means of an Anrep viviperfusion flask to prevent alteration of the circulation volume of the recipient. It was also found necessary to centrifuge the extract to remove fat, and to heparinize the recipient animal, to prevent intravascular clotting.

It was found that perfusion of the total extract from the traumatized limbs invariably caused a slight transient rise of the blood pressure of 8 to 26 mm./Hg. in 5 cases. Administration of the bloody fluid portion alone produced rises of the blood pressure of 7 to 17 mm./Hg. in 4 of 7 cases, while the extract of muscles alone caused transient falls of 5 to 41 mm./Hg. in 5 of 7 cases. Recovery of the blood pressure was very prompt in all cases in which a fall occurred.

It was concluded that: (1) Centrifuged extracts of traumatized limbs obtained by a hydraulic press caused no depression when perfused as a whole in a second animal, but instead caused rises of the blood pressure. (2) Similar extracts of the traumatized muscles alone were slightly toxic, but did not cause a sustained blood pressure fall nor death in any case. (3) The findings fail to support the "toxic theory" of the etiology of traumatic shock.

7699 C

Pregnancy Cells in Rat Pituitary: Influence of Lipoidal Corpus Luteum Extract.

HARRY A. CHARIPPER.

From the Department of Biology, Washington Square College, New York University.

Modifications of the histological picture of the anterior pituitary in the mammal have been shown to be dependent upon extra-hypophyseal somatic changes. The cellular alterations of this gland during pregnancy and following castration are well known. Examination of the anterior hypophysis from clinical cases of hydatidiform moles and of chorio-epitheliomata has demonstrated histological changes similar to those occurring during pregnancy (Rössler,[1] Novak and Koff,[2] Heidrich, Fels and Mathias[3]). Similar effects have been obtained experimentally in the pituitaries of rabbits, guinea pigs, and rats by the injection of foetal and placental extracts (Berblinger,[4] Lehman,[5] Baniecki[6]). Experimental ovarian luteinization by means of the injection of fresh anterior pituitary tissue also has been shown to produce pregnancy changes in the mouse pituitary (Haterius and Charipper[7]).

The histological changes of the anterior pituitary involving the appearance of pregnancy cells, experimentally induced, appear to be related to the presence of some product of gestation. Haterius and Charipper[7] have indicated that the ovary is necessary as an intermediate agent for the production of pregnancy cells in the anterior hypophysis and, furthermore, that in the luteinization of the ovary lies the factor essential for the production of these cells. With the development of a potent lipoidal extract of the corpus luteum it appeared possible to test this hypothesis.

All animals used were sexually mature. The various groups of 5 each were made up of mates from not more than 2 litters of the same age. In all cases, daily vaginal smears were made for several weeks to determine the oestrous cycles. Only those with regular

[1] Rössler, H., *Z. f. Geburtsh. u. Gynäk.*, 1929, **96**, 516.

[2] Novak, E., and Koff, A. K., *Am. J. Obst. and Gynec.*, 1930, **20**, 481.

[3] Heidrich, L., Fels, E., and Mathias, E., *Bruns. Beitr. z. klin. Chir.*, 1930, **150**, 349.

[4] Berblinger, W., *Verhandl. d. dtsch. pathol. Ges., München*, 1914, 184.

[5] Lehman, J., *Virchow' Arch. f. path. Anat. u. Physiol.*, 1928, **286**, 346.

[6] Baniecki, H., *Arch. f. Gynäk.*, 1928, **134**, 693.

[7] Haterius, H. O., and Charipper, H. A., *Anat. Rec.*, 1931, **51**, 85.

normal cycles were used. The animals were divided and given 15 daily injections of 0.5 cc. as indicated in Table I.

TABLE I.

Group No.	Animal	Treatment
1	Normal females	*Lipo-lutin
2	Bilaterally castrated females	"
3	Normal females	Estrogen
4	Bilaterally castrated females	"
5	Normal males	Lipo-lutin
6	Bilaterally castrated males	"
7	Normal males	Estrogen
8	Bilaterally castrated males	"
9	Normal females	Minced brain tissue in normal saline
10	" males	Minced brain tissue in normal saline

* The lipo-lutin and estrogen utilized in this investigation was kindly donated by Parke, Davis and Co. The lipoidal extract of the corpus luteum obtained from this source gave a positive reaction when biologically assayed for deciduomata formation.

At the end of the fifteenth day the animals were all sacrificed and their pituitaries fixed in Guthrie's modification of Zenker's fluid; sectioned in paraffin and stained with Delafield's haematoxylin and eosin. Similarly fixed and stained pituitaries were obtained from untreated pregnant, non-pregnant and castrate females and untreated normal and castrate males.

Histological examination of the anterior pituitaries from the estrogen treated animals and those injected with minced brain tissue in normal saline showed no apparent irregular changes. The cells in every case appeared normal when compared to those from the control animals.

Examination of the anterior pituitaries from animals treated with a lipoidal extract of the corpus luteum were all uniform in their histological appearance when compared one to the other within the respective groups. They all showed the presence of large ovoid cells with a deeply eosin-stained homogeneous cytoplasm surrounding a usually eccentrically placed vesicular nucleus. These cells appear to be identical with those found in the anterior pituitary obtained from pregnant animals. It is of further interest to note that the pituitaries obtained from both male and female castrate animals treated with lipo-lutin contained beside the cell type just described, many small poorly delineated castration cells, indicating a tendency toward the correction of castration cell types in this gland. This phenomenon has been shown, previously, to obtain in male rats receiving ovarian implants (Haterius and Nelson[8]).

[8] Haterius, H. O., and Nelson, W. O., *J. E. Z.*, 1932, **61**, 175.

Summary. Evidence is offered that the essential factor for the production of pregnancy cells in the anterior pituitary of the rat is contained in the luteinized ovary and, further, that this factor is not sex specific.

7700 P

Effects of Thyreotropic and Adrenotropic Hormones on Hypophysectomized Frog Tadpoles.

WAYNE J. ATWELL.

From the Department of Anatomy, University of Buffalo.

Twenty-four specimens of *Rana sylvatica* and *Rana pipiens* hypophysectomized at the tail-bud stage, and kept in the laboratory for 525 days, whose normal mates had metamorphosed at 65-70 days, were divided into 3 groups. Seven animals were kept as controls, 7 were treated with Collip's adrenotropic[1] hormone, batch AI, and the remaining 10 with his thyreotropic[2] hormone, batch TG. As a result of his own bio-assays (on rats) Collip stated that the thyreotropic hormone was not free from adrenotropic material but that the adrenotropic hormone was 'fairly pure'. The hormones were administered by intraperitoneal injection, using special fine needles, in 0.05 cc. doses, given once daily, 6 days a week. For the injection an animal was removed from its aquarium and placed on a pad of absorbent cotton wet with ice water. It was then immobilized, ventral side uppermost, by a smaller sheet of wet cotton having a slit cut in it through which the injection could be made. Following the injection the animal was quickly returned to its aquarium.

In 4 days both of the treated groups were dark in color. The adrenotropic group was decidedly darker than the thyreotropic. The only other change noted in the first 5 days was a slight reduction of the tail fin in a few cases.

On the 6th day the hind legs of one animal in the thyreotropic group appeared to be lengthening and by the 10th day all but 2 of this group showed definite growth of the hind legs. From then on the changes normally seen at metamorphosis appeared rapidly. Forelegs emerged from the 11th day on, the tail shortened, the mouth became a transverse slit, characteristic frog spots (*R. pipiens*) ap-

[1] Collip, Anderson and Thompson, *Lancet*, 1933, **2**, 347.

[2] Anderson and Collip, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 680.

peared and from the 14th day animals were living out of the water. Most of the adrenotropic-treated animals showed little leg development or reduction of body or tail-length, although 2 grew hind legs of considerable size.

The animals were autopsied under the binocular microscope. The thyroid and the hypophysis region were preserved in Bouin's fluid, the gonads, kidneys and adrenals in chromic-osmic. The epithelial hypophysis was invariably entirely absent. The thyroids of the untreated group were very small and the individual vesicles distinctly seen. In the thyreotropic group the thyroids were greatly enlarged, rounded and opaque; no vesicles could be observed. The adrenotropic group showed slight enlargement of the thyroids. A determination of the relative volume of the thyroid for 7 individuals of each group gave the following averages: Untreated silvery, 1; thyreotropic, 6; adrenotropic, 2.

Histologically the thyroids of the thyreotropic group show marked stimulation. There is little colloid present; the epithelium is very tall and mitotic figures are numerous. The vesicles apparently have increased in number by branching and division.

After fixation in chromic-osmic and clearing in chloroform the adrenal glands were visualized due to the blackened lipoids contained in the cells of the cortex. In the untreated group the gland is seen as a faintly darkened irregular cord on the ventromesial surface of the kidney. In the adrenotropic animals it is much larger and very heavily stained. The thyreotropic group also shows decided increase in the lipoids of the adrenal. These observations are confirmed by histological study. Grossly the gonads show no apparent modification.

Thus it is seen that the hypophysectomized frog tadpole is a striking test object for the action of pituitary hormones. This is especially true for the thyreotropic, since the objective signs of metamorphosis are more readily observed than are changes in the basal metabolic rate.

7701 C

A Study of the Etiology of Influenza.

A. R. DOCHEZ, K. C. MILLS AND YALE KNEELAND, JR.

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

In a previous communication[1] we have announced the cultivation of a filterable virus from 2 cases of typical influenza, one occurring in an epidemic of the interpandemic type in 1930 and the other during an outbreak of similar character in 1932. These 2 cultures when inoculated intranasally in human volunteers produced respiratory infections of the general type of the common cold but of considerable severity and with a tendency to greater constitutional reaction than is commonly observed after inoculation of cultures of common cold virus.

In March, 1934, there occurred in a small convalescent home for rheumatic children a local outbreak of influenza. The symptoms of the early cases were almost identical, consisting of malaise followed by fever as high as 104°F. and lasting for 3 days. During the height of the disease there was headache, general aching, nausea and vomiting. This was followed by marked weakness for several days. In the later stages of the epidemic the picture was that of a severe cold with only a slight rise in temperature. The leucocyte count was elevated rather than lowered as in some outbreaks of influenza.

Chick embryo tissue cultures were made from filtered nasopharyngeal washings obtained from 2 of the early cases of the disease. One of these cultures in the 18th generation, 2 months after isolation, was inoculated intranasally into 3 human volunteers under rigid quarantine. The results are presented in the following protocols.

Volunteer 93, a heavy, muscular man with no recent history of respiratory disease was inoculated intranasally with 3 cc. of culture R18. The inoculation was repeated 5 hours later. The next morning, 18 hours after the first inoculation, the subject manifested nasal obstruction, sneezing, mucoid nasal discharge, a productive cough and a slight malaise. Later the malaise increased and was accompanied by headache and pain in the back of the neck. The pharynx became sore and inflamed. There was no fever and the maximum leucocyte count was 9000.

[1] Dochez, A. R., Mills, K. C., and Kneeland, Yale, Jr., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1017.

Volunteer 94, a short, heavy set man with no recent history of respiratory infection, was inoculated intranasally with culture R18 at the same time and in the same manner as Volunteer 93. Subject awoke the next morning, 18 hours after the first inoculation, with slight nasal obstruction, mild headache and soreness of back and shoulders. The symptoms became more pronounced during the day and cough, nasal discharge and general malaise developed. On the second day headache and pain in the back were pronounced and there were dizziness, anorexia, and vomiting. The throat was red and swollen. Most of the day was spent in bed. The symptoms lasted for 6 days, there was no fever, the maximum leucocyte count was 12,100.

Volunteer 95, a rather thin man with no history of upper respiratory infection for 6 months. Inoculated intranasally with culture R18 at the same time and in the same manner as Volunteers 93 and 94. The next morning, 18 hours after inoculation, nasal obstruction was present. There was nasal discharge and productive cough. There was general malaise and severe headache. The tonsils showed a few whitish spots and the pharynx was acutely inflamed. Subject became much worse in the afternoon and went to bed complaining of bad pain in neck and back and of feeling dizzy. The subject vomited throughout the night and the next morning appeared acutely ill. There was marked nausea and retching, the chest felt sore and the cough was pronounced and frequent. The face was flushed and the eyes suffused. The temperature reached 101°F. by mouth and the leucocyte count was 10,900. On the third day the fever had subsided but cough and general malaise were still marked. On the fourth day the subject again became worse, complete anorexia was present and vomiting reappeared. The pharynx was diffusely red. Headache and malaise were vary marked and coughing was severe. By the fifth day all the symptoms had abated markedly and he was discharged from quarantine on the seventh day, feeling weak and still slightly dizzy. The maximum leucocyte count was 12,600. The subject likened the experience to an attack of influenza suffered in Germany in 1918.

From these experiments the conclusion seems justified that a filterable agent has been cultivated from clinical influenza which after being carried for 18 transplants in chick embryo medium produced in experimental subjects a picture similar to that seen in the natural outbreak, in which individual attacks ranged from the appearance of common cold to that of typical clinical influenza. The

leucocytes were elevated rather than lowered, a finding, however, which is quite common in natural outbreaks.

No visible organisms of any kind could be cultivated either aerobically or anaerobically from the cultures used for inoculation. With the exception of occasional contaminating diphtheroid organisms nothing was visible microscopically in the cultures at any time nor was there any recognizable visible change in the medium.